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This is a neuroanatomical and ethological study of the pectines, the primary chemosensory organs of scorpions (Arachnida: Scorpiones). The pectines are paired, ventromedial appendages that brush the substrate as the scorpion walks. This comb-like organ consists of a supportive spine and an array of teeth. Each tooth supports hundreds of setaform sensilla called pegs.

The peripheral neuroanatomy of sensilla on the teeth and spine were described. Below the peg sensilla on the pectinal teeth were three histological layers consisting of dendrites, neuronal somata, and a layer of peripheral synapses. The neuronal cell layer within the teeth was further divided into inner and outer sub-laminae, comprised of chemosensory and mechanosensory neurons. The peg sensilla were innervated by iterative cassettes of sensory neurons orthogonal to the layers described. Sensory hairs on the pectinal spine resembled arachnid tactile and chemosensory hairs.

The central projection patterns of sensory afferents from tactile hairs on the pectinal spine and peg sensilla on the pectinal teeth were compared. The internal

architecture of the neuropile serving the pectines was also characterized. The pectinal neuropile contained a basal disk and a terminal cap. The cap was divided into a fibrous cortex and a medullar region of glomeruli. Sensory afferents from the tactile hairs on the spine were aligned topographically in the pectinal neuropile, but were restricted to the outer cortex. Sensory afferents from the bimodal peg sensilla terminated in both regions of the pectinal neuropil, suggesting that the cortex and medulla are functionally divided into mechanosensory and chemosensory processing areas.

Behavioral experiments were conducted using a Y-maze choice test to determine if male scorpions were capable of trailing female conspecifics. A significant proportion of male scorpions preferred the arm of the Y-maze that a conspecific female had walked down and pre-courtship behaviors were only evoked when male scorpions contacted substrates that had been exposed to a female scorpion. The results from this experiment suggest that scorpions may use chemical cues to find potential mates and to initiate courtship.

The Pectines of Scorpions: Analysis of Structure and Function

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John M. Melville, Author

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Sara K. Tallarovic assisted in collection of animals, data, behavioral assays and experimental design for chapter 4 of this thesis. Anatomical and behavioral studies were conducted in the laboratory of Dr. Philip Brownell. Nick Oesch assisted in neuronal backfilling, tissue preparation, and dissection of scorpion central nervous system preparations presented in chapter 3.

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DEDICATION

This body of work is dedicated to my incomparable partner, wife and colleague Sarah McBride. She has been and always will be a constant inspiration. Without her unflagging support, understanding and determination this thesis would not have been completed.

The Pectines of Scorpions: Analysis of Structure and Function

Chapter 1: Introduction

Overview

This is a neuroanatomical and ethological study of the pectines, one of the primary sense organs of scorpions. The pectines are ventral appendages that detect both chemosensory and tactile information from the substrate and appear to share several organizational features with visual and somatosensory systems (sensory systems that detect features of physical spaces). This dissertation focuses on the central and peripheral neuroanatomy of this unique sensory system and investigates mate trailing, a behavior that may be mediated by the pectines.

Structure and Function of the Pectines

The pectines of scorpions are paired comb-like appendages that extend laterally and ventrally from the mesosoma where they actively brush the substrate (Hjelle, 1990). Each pectine is composed of a supportive spine and a line of teeth that support dense arrays of small (< 5 μm) setaform sensilla called pegs (Carthy, 1968; Swoveland, 1978; Hjelle, 1990).

The function of this organ has remained enigmatic for more than a century. Previous investigators speculated that the pectines were tactile, olfactory, or vibration detectors while others believed they were used in respiration, reproduction, and birthing (Carthy, 1968; Root, 1990 for review). The majority of recent studies have supported Schroder's hypothesis (1908) that scorpions use the pectines to monitor the mechanical and chemical properties of substrates.

For almost half a century, the detection of substrate texture was considered the exclusive function of the pectines. A series of behavioral experiments by Alexander (1957; 1959) and Abushama (1964) demonstrated that scorpions use the pectines to find suitable substrates for spermatophore deposition and preferred habitats. Subsequent anatomical and physiological studies suggested that the peg sensilla were purely mechanoreceptive and likely mediated both of these behaviors. Apical pores typical of arthropod chemosensory hairs (Slifer, 1970) were not found on the peg sensilla (Carthy, 1966; 1968) and only deflection of the sensillum shaft produced action potentials in the pectinal nerve (Hoffman, 1964). Application of chemical, olfactory, or aqueous stimuli to the peg sensilla was not associated with neural activity (Hoffman, 1964).

Two separate ultrastructural studies by Ivanov and Balashov (1979) and Foelix and Muller-Vorholt (1983) were the first to demonstrate that the peg sensilla resembled other arachnid chemosensory hairs (Foelix and Chu-Wang, 1973b). Each peg sensillum had a slit-like terminal pore and was innervated by two classes of dendrites. One dendrite terminated at the base peg of the sensillum, resembling

a mechanoreceptor. The remaining dendrites terminated near the apical pore, resembling chemoreceptors (Ivanov and Balashov, 1979; Foelix and Muller-Vorholt, 1983; Foelix, 1985 for review). The peg sensilla were subsequently shown to respond to a wide range of chemicals, including alkanes, alcohols, aldehydes, esters, carboxylic acids, ketones, and water (Gaffin and Brownell, 1990, 1997a).

Behavioral studies suggest that the pectines detect substrate borne chemical cues from prey and conspecifics. Scorpions appear to locate buried prey items after the pectines contact sand treated with prey extracts (Krapf, 1986). Pectinal sweeping activity increases when male scorpions contact sand that has been previously occupied by a female conspecific (Krapf, 1986; Gaffin and Brownell, 1992; Tallarovic, 2000). This increase in substrate sampling also occurs when male scorpions are exposed to substrates treated with hexane or methylene chloride washes from the cuticle of female scorpions, suggesting that the pectines detect female sex pheromones (Gaffin and Brownell, 1992).

The chemosensory pectines share certain organizational features with visual and somatosensory systems (sensory systems that encode properties of a physical space). The surface of the pectinal teeth contains a dense array (15,000-25, 000 sensilla / mm^2) of uniform sensilla (Swoveland, 1976) that are capable of detecting substrate features an order of magnitude smaller than a sand particle (average diameter of a sand grain =150-200 μm)(Brownell, 2000). Sensory neurons innervating the peg sensilla form peripheral axonic synapses (Foelix and Muller-

Vorholt, 1983) that may be involved in sensory processing (Gaffin et al., 1991; Gaffin and Brownell, 1997b) and afferents from the pectinal teeth project topographically to the central nervous system (Brownell, 1989; 1998). This pattern of neural organization suggests that the pectines may be capable of high resolution 'imaging' of both substrate texture and chemistry.

The peripheral organization of sensory neurons innervating the peg sensilla has not been described. The majority of studies have focused on the ultrastructure and physiology of the peg sensilla (Hoffman, 1964; Ivanov and Balashov, 1979; Foelix and Muller-Vorholt, 1983; Gaffin and Brownell, 1990; 1997a,b). Several studies have defined the gross anatomy of the scorpion central nervous system (Hanstrom, 1923; Babu, 1965; 1985 for review) but the central organization of this sensory system has been investigated only recently (Brownell, 1998). This is surprising given that Gaskell (1902) and Schroder (1908) first investigated the gross histology of the pectines almost 100 years ago.

Natural History and Sensory Ecology of Sand Scorpions

The perceptual ability of an animal is often uniquely adapted to its ecological 'niche' (Dryer and Brockman, 1996 for review). A crucial step in developing insight into the function of a sensory system requires a complete understanding of the natural history and ecology of the organism under study (Roeder, 1963; Dryer and Brockman, 1996 for review). The behavioral function of

this chemosensory organ is unknown. A brief review of the natural history of desert scorpions is provided for this purpose.

Scorpions are abundant and diverse in desert ecosystems (Hibner, 1971, Polis, 1990) are long-lived (5-30 years depending on species) and are some of the largest terrestrial arthropods (Polis and Sissom, 1990). Desert scorpions are solitary and nocturnal animals that have a home range of less than a meter in radius and live in burrows that require substantial energetic output to construct and maintain (Polis, 1990). Desert scorpions are typically ambush predators that specialize on insects and other scorpions, including smaller conspecifics (Polis, 1979; Polis and McCormick, 1987; McCormick and Polis, 1990 for review).

Desert scorpions only mate in the late summer months when temperatures are moderate. During this period, male scorpions often leave their burrows to 'wander' dune environments, presumably in search of female conspecifics (Polis and Farley, 1980). This wandering behavior increases male predation risk (Polis and Farley, 1979; Polis and Sissom, 1990). The surface densities of scorpions during this brief mating season are relatively high (4-40 individuals per ha depending on the species) but only a fraction of the animals on the surface are reproductive (Polis, 1990). The selective pressure associated with mate finding and predator avoidance suggests that undirected mate searching by male desert scorpions would be maladaptive.

The role that sex pheromones may play in mate-finding in scorpions is not clear, but sexual communication in other arachnids may provide some useful clues. Spiders use both volatile and contact sex pheromones to find mates and mediate courtship (Schultz and Toft, 1993; Hegdekar and Dondale, 1969; Suter and Renkes, 1982; Trabalon et al., 1997). Spider sex pheromones are located in the silk (Roland, 1984; Suter and Hirscheimer, 1986) and possibly the cuticle of female conspecifics (Suter et al., 1987; Trabalon et al., 1997; Prouvost et al., 1999). Male spiders will actively trail female drag-lines (Tietjen, 1977; Tietjen and Rovner, 1980; Taylor, 1998) and exhibit courtship behaviors after contacting female webs or substrates previously occupied by female conspecifics (Jackson, 1987; Barth and Schmitt, 1991; Prouvost et al., 1999). Female scorpions produce specular trail-like deposits that could serve a similar function (Brownell, pers obs) but mate-trailing has not been investigated in scorpions.

Scorpions may use sex-pheromones to initiate courtship (Krapf, 1986; Gaffin and Brownell, 1992; Tallarovic, 2000). When male scorpions encounter the home range of a female conspecific, a suite of behaviors are often evoked in the male that appear to subdue the cannibalistic tendencies of the female (Polis and Farley, 1979). Juddering and tail-wagging are two pre-courtship behaviors described as high frequency oscillations of the main body and shaking of the aculeus or 'sting' (Gaffin and Brownell, 1992; Tallarovic, 2000). Male desert scorpions exhibit juddering and tail-wagging when exposed to substrates previously occupied by female conspecifics or sand treated with hexane washes from the

cuticle of female scorpions (Krapf, 1986; Gaffin and Brownell, 1992). These behaviors may be 'released' by substrate borne chemical cues (sex pheromones) (Gaffin and Brownell, 1992; Gaffin and Brownell, 2000 for review).

Statement of Purpose

The objective of this study was to describe the peripheral and central organization of the pectinal sensory system and to conduct behavioral experiments that would elucidate the behavioral function of this unique sensory organ.

The second chapter of this dissertation focuses on the peripheral cytoarchitecture of the pectinal teeth. It includes a description of how sensory neurons innervating the peg sensilla are organized, and investigates the location and morphology of a plexus of peripheral synapses discovered at the ultrastructural level by Foelix and Muller-Vorvolt (1983).

The third chapter focuses on the central organization of the pectinal sensory system. It includes a comparison of the central projection patterns of sensory afferents from tactile hairs on the pectinal spine and the bimodal (chemosensory and mechanosensory) peg sensilla on the pectinal teeth. The neuropile serving the pectines is also characterized. The desert sand scorpion (*Paruroctonus mesaensis*) was the research organism for both anatomical studies. This species has proven to be an exceptional preparation for investigating arachnid chemoreception at the

anatomical and physiological level (Brownell, 1989; 1998; Gaffin and Brownell, 1990, 1997a,b).

The fourth chapter investigates mate trailing in scorpions, a sensitivity that may be mediated by the pectines. The objectives of this study were to determine the following: 1) are male scorpions capable of trailing female conspecifics, 2) can males scorpions discriminate female trails from trails of male conspecifics and 3) how is female substrate exposure related to trailing efficiency, locomotion, and behavior of male scorpions. The giant hairy desert scorpion (*Hadrurus arizonensis*) was the research organism for this behavioral study. This organism is a potential model system for investigating pheromonal signaling in scorpions: male and female *H. arizonensis* will court and mate in the laboratory (Tallarovic, 2000) and can be assayed for reproductive behavior in a manner of minutes (Melville et al., 1999; Tallarovic, 2000). In addition, sex-specific courtship behaviors are easily discernable from conspecific aggressive behaviors (Tallarovic, 2000).

Background Information

All animals use cues from the external world to direct their behavior. Sense organs gather and present information to the central nervous system. Sensory systems extract different qualities, features, and patterns of information from the environment that are ultimately related to the survival and reproductive success of the organism (Roeder, 1963; Wehner, 1987; Heiligenberg, 1991; Dusenberry, 1993). For many animals, determining the nature of the stimulus, where it is

located, and when it occurs are of central importance. Sensory systems accomplish these tasks by detecting different spatial, temporal, and spectral characteristics of sensory signals (Heiligenberg, 1991; Dusenberry, 1993). The following section focuses on the organization of tactile, olfactory, and gustatory systems of arthropods. Where appropriate, features of vertebrate sensory systems will be used to exemplify organizational themes.

Sensory afferents from the mechanosensory sensilla of many insects project somatotopically into the central nervous system, forming spatially organized receptor maps (Ghysen, 1980; Murphey, 1981; Johnson and Murphy, 1985; Levine et al., 1985; Kent and Levine, 1988; Peterson and Weeks, 1988; Newland, 1991b). The tactile hairs on the legs of the locust (*Schistocerca gregaria*) and the cercal sensory system of the cricket (*Acheta domesticus*) are two examples.

The walking legs of locusts support sparse populations (< 200) of sensory bristles involved in reflexive actions (Pfluger, 1980). Each bristles plane of movement is constrained by a cuticular socket to the long axis of the leg. The mechanoreceptor innervating each bristle responds maximally to proximal deflections through this axis (Newland, 1991a). Deflection of a single bristle produces a coordinated movement of the leg (Pfluger, 1980). The movement of the leg is directly related to the location of the stimulus. When the lateral side of the femur is touched the leg moves medially. When the ventral surface is touched the leg is raised (Pfluger, 1980; Siegler and Burrows, 1986).

Sensory axons from the tactile bristles project somatotopically into the thoracic ganglion, creating a three-dimensional map of the legs surface in the ventral neuropil (Pfluger et al., 1988; Newland, 1991b). A series of midline spiking interneurons in the thoracic ganglion receive monosynaptic input from the sensory bristles (Siegler and Burrows, 1983; Nagayama and Burrows, 1990). The receptive field of each interneuron is directly correlated with the position of its dendritic arbor within this neural map (Siegler and Burrows, 1986; Burrows and Newland, 1993). These midline spiking interneurons are involved in polyneuronal circuits that drive the musculature of the leg (Burrows and Siegler, 1982), suggesting that the nervous system of the locust uses tactile sensory maps to produce appropriate reflexive actions (see Burrows, 1996 for review).

The cercal system of the cricket (*Acheta domesticus*) is another example of a spatially organized mechanosensory system (Jacobs and Theunissen, 1996; 2000). The cerci are sensory appendages that crickets use to detect potential predators (Gnatzy and Kamper, 1990; Olberg and Miller, 1991). The paired cerci are located on the terminal segment of the animal and support a variety of mechanosensory sensilla, including 1000-2000 filiform hairs that are displaced by near field air currents (Chiba et al., 1992; Landolfa and Miller, 1995). The cuticular socket of each filiform hair constrains the sensillar shaft to (L) longitudinal, (O) oblique, or (T) transverse deflections relative to the longitudinal axis of the cercus (Gnatzy and Tautz, 1980). The mechanoreceptor innervating each hair responds maximally to a single air current direction within this movement plane (Edwards and Palka, 1974;

Landolfa and Miller, 1995). Different classes of directionally sensitive hairs (T-hairs, L-hairs, and O-hairs) are distributed in a non-uniform fashion along the length of the organ, producing an array of receptors that sense all possible wind current directions (Tobias and Murphey, 1979; Bacon and Murphey, 1984; Walthall and Murphey, 1986; Landolfa and Jacobs, 1995; Roddy and Jacobs, 1996).

Sensory axons from the filiform hairs project in orderly arrays to a specialized glomerulus in the terminal ganglion, creating a continuous three-dimensional representation of air current direction (Bacon and Murphey, 1984; Walthall and Murphey, 1986; Jacobs and Nevin, 1991; Troyer et al., 1994, Jacobs and Theunissen, 1996). Axons from the filiform hairs make monosynaptic connections on a series of identifiable interneurons in the terminal ganglion that are 'tuned' to different wind directions (Bacon and Murphey, 1984; Jacobs et al., 1987; Miller et al., 1991). The directional 'tuning' of each interneuron is directly related to the position of its dendritic arbor within this neural map (Miller et al., 1991; Troyer et al., 1994; Paydar et al., 1999; Jacobs and Theunissen, 2000).

Analogous arachnid mechanosensory systems are also organized somatotopically (Babu and Barth, 1989; Anton and Barth, 1993; Gorb et al., 1993). In spiders, cuticular hairs on the ventral surface of each leg mediate a body-raising reflex (Eckweiler and Seyfarth, 1988; Hoger and Barth, 1995). Deflection of just one of these 'tactile' hairs produces a coordinated extension of all eight legs that raises the body above the point of contact (Eckwieler and Seyfarth, 1988; Hoger

and Barth, 1995). Each tactile hair is innervated at its base by three sensory neurons (Foelix and Chu-Wang, 1973a). All of these neurons fire action potentials when the shaft of the hair is deflected proximally or distally along the long axis of the leg (Harris and Mill, 1977a; Foelix, 1985a). Specialized hair-like or racket shaped sensilla called trichobothria are also found on the appendages of many arachnids (Reissland and Gorner, 1985). These sensilla are deflected by near field air currents and are used by spiders to sense the air vibrations of flying insects (Reissland and Gorner, 1985; Barth et al., 1993; 1995; Barth and Holler, 1999; see Barth, 2000 for review). The supportive cuticular cup (bothrium) of each trichobothria limits the deflection angle of the sensillar shaft to approximately 35 degrees in any given direction (Barth et al., 1993). In spiders, each trichobothrium is innervated at its base by four sensory neurons (Reissland and Gorner, 1985). Three of these neurons are uniquely directionally sensitive: each neuron only responds when the shaft of the hairs is deflected distally, laterally or medially (Gorner, 1965; Harris and Mill, 1977a).

The arachnid subesophageal nerve mass is a plurisegmental ganglion, containing a pair of neuromeres for each appendage (Babu, 1985; Babu and Barth, 1989). Each neuromere superficially resembles a ganglion, consisting of an outer rind of somata and a central neuropile. A series of transverse and longitudinal tracts connect all the neuromeres of the subesophageal nerve mass (Babu, 1985). The longitudinal tracts run along the anterior-posterior axis of the subesophageal nerve mass (SOM) and are divided into dorsal and ventral tracts of fibers (Babu,

1985). The dorsal tracts consist of larger diameter fibers from motorneurons and interneurons. The ventral tracts consist of smaller axons from sensory afferents (Babu, 1985). Many arachnid sensory axons have bistratified terminals: a smaller arbor of terminals is found in the neuropil of the associated neuromere, but the primary arbor is located in one of the six VLTs of the SOM (Babu and Barth, 1989; Anton and Barth, 1993; Gorb et al., 1993). These ventral longitudinal tracts (VLTs) are considered one of the primary sensory integration centers of the arachnid brain (Babu and Barth, 1989; Gronenburg, 1990).

Axons from sensory neurons innervating the tactile hairs and trichobothria are aligned somatotopically in the ventral longitudinal tracts (Anton and Barth, 1993). Sensory afferents from sensilla located on the tip of an appendage terminate ventrally in the VLTs and sensilla located proximally have axons that terminate more dorsally in the VLTs (Anton and Barth, 1993; Gorb et al., 1993). A series of interneurons in the supraesophageal ganglion respond to different types of tactile stimuli. Dendrites from these neurons project into the VLTs, but their arbors have not been well characterized or correlated with their response properties (Gronenburg, 1989; 1990; Friedel and Barth, 1997).

Visual systems are also spatially organized. The optic lobes of flies consist of four successive neuropils (lamina, medulla, lobula, and lobula plate) that are all organized retinotopically (Strausfeld, 1976; Strausfeld and Lee, 1991). Three parallel pathways within this retinotopic architecture process different types of visual information (color, achromatic contrast, movement, and stimulus direction)

(Strausfeld and Lee, 1991; Bausenwein and Fischbach, 1992; Douglass and Strausfeld, 1996, 1998; Anderson and Laughlin; 2000).

The compound eyes of insects are composed of a two-dimensional array of ommatidia that both gather and sense light (Horridge, 1977; Young, 1989; Caveney, 1998 for review). In flies, each ommatidium is composed of an external facet, a pseudocone, and the light sensing membranes (rhabdomes) of eight different photoreceptors (R1-R8) (Hardie, 1985; 1986; Anderson and Laughlin; 2000). Photoreceptors R1-R6 are excited by a broad spectrum of wavelengths (350 nm to 510 nm). Photoreceptors R7-8 sense a narrower spectrum of ultraviolet and or green wavelengths and different planes of polarized light (Hardie and Kirschfeld, 1983; Hardie, 1985; 1986; Anderson and Laughlin, 2000).

The axons from photoreceptors in each ommatidium form retinotopic projections that anatomically reconstruct the visual world of the fly (Kirschfeld, 1967; Strausfeld, 1976; 1989). Because the surface of the compound eye is curved, photoreceptors in neighboring ommatidia sample overlapping points in the visual field (Kirschfeld, 1967; Land, 1985). Axons from all eight photoreceptors (R1-R8) sampling the same point in different but adjacent ommatidia converge, projecting in two separate pathways to the lamina and medulla of the optic lobes (Trujillo-Cenoz, 1965; Braitenberg, 1967, Kirschfeld, 1967, see Caveney, 1998 for review).

The axons from photoreceptors R1-R6 project directly to the lamina, forming retinotopic columns of synaptic cartridges (Campos-Oretga and Strausfeld 1973; Strausfeld and Campos-Oretga, 1977; Shaw, 1989; Strausfeld and Lee,

1991). Each cartridge contains the primary neurite of 3-5 large monopolar cells (L1-5). Monopolar cells L1-3 are the primary output cells of the lamina (Strausfled, 1976; Trujillo-Cenoz, 1985). The primary neurite of L3 is located on the edge of each cartridge, receiving less than 12% of the available synaptic contacts (Strausfeld and Lee, 1991). In contrast, the axons of photoreceptors R1-R6 make the majority of their contacts (> 300 synapses) with the neurites of L1-L2. These two monopolar cells (L1-L2) also receive synaptic input from over twelve different classes of tangential and centrifugal cells in the lamina (Strausfeld and Campos-Oretga 1973; 1977; Strausfeld and Lee, 1991) that are likely involved in neural adaptation and producing the on-center off-surround response observed in L1-L2 at high light levels (Jarvilehto and Zettler, 1972; Laughlin and Hardie, 1978; Dubs, 1981; Laughlin, 1989; Anderson and Laughlin, 2000).

Visual information from photoreceptors R1-R6 and R7-R8 converge in the medulla of the optic lobes (Strausfeld, 1976; 1989). Axons from the large monopolar cells (L1-3) leave the lamina, decussate, and project to the medulla, forming retinotopic columns of terminals (Strausfeld, 1976; Trujillo-Cenoz, 1985; Strausfeld, 1989). The axons of photoreceptors R7-8 project directly to the medulla (Cajal and Sanchez, 1915; Melamed and Trujillo-Cenoz, 1968), converging with the axons of large monopolar cells (L1-3) that receive information from the same point in the visual field (Strausfeld and Lee, 1991). Each retinotopic column supports two populations of terminals. The axons of L1-L2 arborize

superficially in each column. The axons of L3 and R7-8 project deeper into the medulla and have overlapping terminal fields (Strausfeld and Lee, 1991).

The lobula receives retinotopic projections from the medulla and may be involved in the discrimination of colors (Strausfeld and Lee, 1991; Gilbert and Strausfeld, 1992; Strausfeld and Gilbert, 1992; Douglas and Strausfled, 1998). Each column of terminals in the medulla contains the primary neurites of a heterogeneous population of output (transmedullary) neurons. The terminals of L3 and R7-8 overlap with the dendrites of a class of wide field transmedullary (wTM) neurons that project to the lobula retinotopically (Strausfeld and Lee, 1991; Strausfeld and Gilbert, 1992). Neurons within the lobula of the honey bee (*Apis melifera*) respond to different colors of stimuli (Hertel, 1980; Hertel and Maronde, 1987), suggesting that the lobula is involved in chromatic processing (Strausfeld and Lee, 1991; Douglass and Strausfeld, 1998).

The lobula plate also receives retinotopic projections from the medulla (Strausfeld, 1976). This neuropil appears to be involved in flight stabilization, optomotor responses, and other behaviors that depend on visual motion processing (Eckert and Bishop, 1978; Hausen, 1984; 1993; Douglas and Strausfled, 1995; 1996; Laughlin, 1999). The terminals of L1-L2 overlap with two different classes of transmedullary cells that project retinotopically into the lobula plate (Buschbeck and Strausfeld, 1996; 1997). Type 1 transmedullary cells (Tm1) project to a superficial layer of the lobula. Their terminals overlap with the neurites of a class of neurons called T5 bushy cells (Buschbeck and Strausfeld, 1996; 1997). The T5

busy cells are directionally sensitive and terminate in successive layers of the lobula plate (Douglas and Strausfeld, 1996), supplying horizontal and vertically sensitive wide field neurons (Eckert and Bishop, 1978; Single and Borst, 1998; Krapp et al., 1999). Sets of intrinsic transmedullary cells (iTm) also have dendrites that overlap with the terminals of the L1-L2. The axons of these iTMs terminate at the base of the medulla and overlap with the dendrites of a class of bushy T4 cells (Buschbeck and Strausfeld, 1996; 1997). The T4 cells project back into the lobula plate and respond to motion and flicker, but are not directionally sensitive (Douglas and Strausfeld, 1995; 1996). This achromatic visual pathway may be involved in determining flight velocity, distance, and other parameters that do not rely on directional information (Srinivasan et al., 1993; Douglass and Strausfeld, 1996).

Arthropods use specialized olfactory appendages to detect a diverse assemblage of chemicals in aerial and aquatic environments (Dusenberry, 1993 for review). Chemical signals that are species and even sex specific are also used in intraspecific communication. Many insects can detect subtle structural alterations in these olfactory signals (Mustaparta et al., 1980; Hansen, 1984; Leal and Mochizuki, 1993; Larsson et al., 1999; see Hannson and Anton, 2000 for review). Determining how olfactory systems encode qualitatively diverse forms of information while maintaining such a high level of specificity is a central question in the field of sensory biology (Shephard, 1994; Hildebrand and Shephard, 1997; Laurent, 1999).

The primary olfactory organs of mandibulate arthropods (insects and crustaceans) are the antennae. These jointed appendages are found on the head of the animal where they actively sample the environment. The antennae of mandibulate arthropods support several different classes of multi-porous sensilla that are innervated by groups of odor sensitive neurons (Kaissling, 1986; Derby and Atema, 1988). The axons of theses olfactory receptor neurons project to a region of the arthropod brain called the antennal lobe, terminating in several hundred spheroid (insects) or cone shaped regions (crustaceans) of neuropil called glomeruli (Mellon and Munger, 1990; Boeckh and Tolbert, 1993; Schmidt and Ache, 1996).

Central olfactory processing areas in vertebrates and insects are organized in a similar fashion (Hildebrand and Shepard, 1997). The olfactory epithelium is the primary olfactory organ of vertebrates. Water or air is brought through the nares and across the olfactory epithelium. This sensory epithelium supports many olfactory receptor neurons that respond to different odorants (Gesteland et al., 1965; Firestein and Werblin, 1989). Axons from the olfactory receptors neurons project to a region of the vertebrate brain called the olfactory bulb, terminating in spheroid glomeruli (Shephard, 1994; Hildebrand and Shephard, 1997 for review).

The glomeruli may be modular olfactory processing units that detect different molecular features of odorants (Shepard, 1994; Hildebrand and Shephard, 1997; Mori et al., 1999 for reviews). This hypothesis is supported by neuroethological studies of pheromonal signaling in insects (Hildebrand, 1995; Hannson and Anton, 2000 for review). Many female moths release a specific blend

of chemicals into the air that serve as sex pheromones (Linn et al., 1984; 1986). Each component of the pheromone blend is detected by a separate type of sensory neuron located in multi-porous trichoid sensilla on the antennae of the male (Kaissling, 1986; Kaissling et al., 1989). The antennal lobe of the male contains a set of sexually dimorphic glomeruli called the macroglomerular complex (MGC)(Sanes and Hildebrand, 1976). Each glomeruli of the MGC receives terminals from only one type of pheromone sensitive neuron (Hannson et al., 1991; 1992). Female moths process olfactory signals involved in oviposition and egg-laying behaviors in another set of sexually dimorphic glomeruli in the antennal lobe (King et al., 2000).

Many odors are complex mixtures of naturally occurring chemicals, not sex pheromones (Shephard, 1994; Hildebrand and Shephard, 1997; Mombaerts, 1999 for review). If each glomerulus detects a different molecular feature, then general odors could be discriminated by the specific pattern of activity each odor elicits across the array of glomeruli (Leveteua and Macleod, 1966; Hildebrand and Shephard, 1997; Mombaerts, 1999; but see Laurent, 1999). This model of olfactory discrimination is supported by molecular studies in both vertebrates and insects.

Putative olfactory receptors in vertebrates belong to a diverse multi-gene family (>1000 genes) of seven transmembrane G-protein coupled receptors (Buck and Axel, 1991; see Mombaerts, 1999 for review) that appear to bind different odorants (Zhao et al., 1998; Malnic et al., 1999). Only one or a few types of

olfactory receptor proteins are expressed by each olfactory receptor neuron (Lancet, 1986; Shephard and Firestein, 1992; Chess et al., 1994; Malnic et al., 1999). All olfactory receptor neurons expressing the same receptor protein converge onto a single glomerulus in the olfactory bulb (Vassar et al., 1994; Mombaerts et al., 1996; Mombaerts, 1999 for review). The functional significance of this glomerular convergence is unknown but suggests that odor identity could be discriminated by the defined spatial and temporal pattern of activity evoked by an odorant across all the glomeruli (see Hildebrand and Shephard, 1997; Mori and Yoshihiro, 1999 for review). This model is further supported by activity dependent studies using voltage activated dyes and single unit recording analysis: different sets of odors activate stereotyped sets of glomeruli in the olfactory bulb (Cinelli and Kauer, 1994) and mitral and tufted cells innervating neighboring glomeruli in the olfactory bulb respond to related structural odorants (Mori et al., 1992; Imamura et al., 1992; Mori and Yoshihiro, 1999 for review).

The glomeruli of the antennal lobes may also detect different molecular features of odorants (Vosshall et al., 2000; Gao et al., 2000). In *Drosophila*, candidate olfactory receptors proteins are expressed exclusively in olfactory sensilla located on the maxillary palps and third antennal segment (Clyne et al., 1999; Gao and Chess, 1999; Vosshall et al., 1999). These candidate olfactory receptors belong to a highly variable family of 60 genes that all have a characteristic seven-transmembrane spanning region (Clyne et al., 1999; Gao and Chess, 1999; Vosshall et al., 1999; Rubin, 2000; Vosshall et al., 2000; Gao et al.,

2000). Only one or a few receptor genes are expressed in any given olfactory receptor neuron and olfactory receptor neurons expressing the same odorant receptor converge on the same glomerulus in the antennal lobe (Vosshall et al., 2000; Gao et al., 2000). Activity mapping (using 2-deoxyglucose) and calcium imaging of the antennal lobes of fruit flies (*Drosophila melanogaster*) and honeybees (*Apis melifera*) also suggest that different odorants activate discrete sets of glomeruli in the antennal lobe (Rodrigues and Buchner, 1984; Rodrigues, 1988, Joerges et al., 1997; Galizia et al., 1999; Sasche et al., 1999).

Olfactory systems appear to be organized to detect the chemical composition of an odor, not the spatial location of individual molecules in the environment (Dusenberry, 1993). This may be related to the diversity of chemical structures that animals can detect in the environment and the physical nature of olfactory signals. Olfactory signals are stochastic, occurring in discontinuous filaments called plumes (Murlis and Jones, 1981; Elkington and Carde, 1984; Dusenberry, 1993 for review). The position of an odor molecule in a plume does not contain inherent spatial information about the location of its chemical source (Carde, 1984; Carde and Baker, 1984; Bell et al., 1995). Most animals appear to locate olfactory signals by detecting concentration gradients or simply follow an odorant upwind to its source (Kennedy et al., 1980, Kennedy, 1983, Schneider, 1992; Bell et al., 1995). The stochastic nature of olfactory signals and apparent lack of spatial information within chemical plumes may explain why somatotopic

projections maps are lacking in the olfactory systems of arthropods (Mellon and Munger, 1990; Christensen et al., 1995).

Arthropod gustatory systems are somatotopically organized (Murphey et al., 1989; Edgecomb and Murdock, 1992; Newland et al., 2000) and reduce a potentially limitless number of chemical stimuli into four or five behaviorally relevant categories (Dethier, 1955; 1976).

The gustatory sensilla of insects are bimodal receptors. Each sensillum is innervated by a set of 3-4 chemosensory neurons and a single mechanosensory neuron (Wolbarscht and Dethier, 1958; Dethier, 1976; Falk et al., 1976; Nayak and Singh, 1983). Insect gustatory sensilla have a single terminal pore and are often short and blunt (sensilla basiconica) or long and hair-like (sensilla trichodea) (Pollack and Balakrishnam, 1997; Singh, 1997; Mitchel et al., 1999). Both types of sensilla are found on the mouthparts, limbs, and body surface of insects, including the wings and ovipositors (Slifer, 1970; Klein, 1980; Chapman, 1982; White and Chapman, 1990)

Insect gustatory neurons are broadly 'tuned' to diverse and often structurally dissimilar chemicals (Singh, 1997; Pollack and Balakrishnam, 1997). The chemosensory neurons within each gustatory sensillum can be classified by their chemical response profiles (Dethier, 1976). The (S) or sugar neuron responds best to a broad range of carbohydrates (e.g. trehalose, pyranose, furanose, galactose, sucrose, and in *Musca* lactose). Both behavioral and pharmacological studies suggest that each carbohydrate has its own receptor site on the S neuron

(Shimada et al., 1974; 1979; Tanimura and Shimada, 1981; Wieczorek et al., 1988; Wieczorek and Wolf, 1989; Schnuch and Seebauer; 1998). Action potentials are also elicited in S neurons when certain amino acids and cyclic nucleotides are applied to the tip of gustatory hairs (Shiraishi and Kuwabara, 1970; Goldrich, 1973; Shimada and Isono, 1978; Shimada et al., 1985; Amakawa et al., 1992) suggesting that several different classes of taste receptors are expressed by sugar sensitive neurons (Ozaki et al., 1993; see Pollack and Balakrishnam, 1997 for review). The (W) neuron responds best to water, but is also excited by several carbohydrates and inhibited by sucrose (Wieczorek and Koppl, 1978; Wieczorek, 1980; Rodrigues and Siddiqi, 1981; Wieczorek et al., 1988). The'classic' salt receptor or (L1) neuron responds best to sodium ions and other non-specific cations. Two different channels or receptors on the dendrite of L1 appear to mediate this dual sensitivity (Gillary, 1966; Siddiqi et al., 1989). Chemosensory hairs on the tarsus of many insects contain a fifth cell that responds to acids and high concentrations of sodium chloride (McCutchan, 1969a,b; Dethier, 1976; Singh, 1997).

Current molecular studies in *Drosophila* also suggest that individual gustatory neurons express several different classes of taste receptors. Putative taste receptors in the fruit fly (*Drosophila melanogaster*) are only expressed in gustatory hairs. These candidate taste receptors belong to a diverse family of 75 –100 genes that do not appear to share significant sequence homologies with other odorant receptors (Clyne et al., 2000). A trehalose (*Tre*) receptor has recently been identified in *Drosophila* (Ishimoto et al., 2000). This putative taste receptor is also

only expressed in gustatory hairs and belongs to a large family of G-protein coupled receptors. Sugar sensitive (S) neurons in the labellar hairs of *Tre* null-mutants are insensitive to trehalose but respond normally to other carbohydrates. Behavioral sensitivity to trehalose is also markedly reduced in flies lacking this gene (Ishimoto et al., 2000).

It is not clear how different classes of gustatory stimuli are represented in the central nervous system of arthropods. In flies, axons from chemosensory hairs on the labellum project to an area of the anterior subesophageal ganglion (SOG) that is composed of seven glomeruli (Stocker and Schroderet, 1981; Shanbhag and Sing, 1992; Singh, 1997). Gustatory axons arborize in several of these glomerular regions and are classified into seven different 'types' of afferents based on the morphology of their terminal arbors (Nayak and Singh, 1985). Different 'types' of afferents are labeled when the labellar hairs are damaged and stimulated with different classes of stimuli (solutions of sugar, salt, or water containing neuronal tracers), suggesting that each 'type' of afferent may belong to a different type of chemosensory neuron (Shanbhag and Singh, 1992). The cellular identity of these labellar afferents remains uncertain because chemosensory neurons respond to such broad and mixed classes of stimuli (Pollack and Balakrishnam, 1997, see Mitchel et al., 1999). Several interneurons within the anterior SOG of the flesh fly *Neobellieria (Sarcophaga) bullata* receive direct chemosensory input from gustatory neurons on the labellum (Mitchell and Itagaki, 1992). A few of these interneurons respond to just one or a few types of gustatory stimuli (sugar, salt, and

water), but the morphology of their dendritic arbors within the SOG have not been characterized (Mitchell et al., 1999). In contrast to the labellar system of flies, chemosensory axons from gustatory sensilla on the legs of flies and locusts are not classified morphologically (Murphey et al., 1989; Newland et al., 2000).

It is clear that gustatory systems of flies are organized topographically and that chemosensory and mechanosensory information is processed in separate regions of the central nervous system (Pollack and Balakrishnam, 1997, but see Mitchel et al., 1999). Axons from the gustatory hairs of flies are aligned in topographic register in modality specific regions of the CNS (Murphey et al., 1989, Yeltmann and Polack, 1986; Edgecomb and Murdock, 1992). Sensory afferents from gustatory hairs on the tarsus of *Drosophila* and *Phormia* project topographically into two different regions of the ventral leg neuropil (VLN). Thinner 'chemosensory' axons project into the medial region of the VNL and thicker 'mechanosensory' axons project into the surrounding outer regions (Murphey et al., 1989; Edgecomb and Murdock, 1992). Axons from tactile hairs on the legs also project topographically into the outer regions of the VNL, suggesting that the ventral neuropil of each leg is divided into chemosensory and tactile processing areas (Murphey et al., 1989; Edgecomb and Murdock, 1992). Sensory afferents from labellar hairs also project topographically into modality specific regions of the SOG (Edgecomb and Murdock, 1992). Thinner "chemosensory" axons terminate in the anterior SOG while thicker

"mechanosensory" axons terminate more medially (Yeltmann and Polack, 1986; Edgecomb and Murdock, 1992; see Mitchell et al., 1999 for review).

Tactile and chemosensory processing areas may not be anatomically segregated in the nervous systems of all insects (Mitchell et al., 1999; Newland et al., 2000). Sensory afferents from gustatory sensilla on the legs of locusts are organized somatotopically, but do not appear to form modality specific projections in the central nervous system (Newland et al., 2000). A position dependent leg withdrawal reflex is elicited when acid vapors or concentrated salt solutions are applied to basiconic sensilla on the tarsus or femur of a locust (Newland et al., 1998; Rogers and Newland, 2000). Midline spiking interneurons involved in tactile reflexes of the leg also receive direct mechanosensory and chemosensory input from the basiconic sensilla (Newland and Burrows, 1994; Newland et al., 1999), suggesting that these interneurons are involved in chemosensory reflexes. Sensory afferents from basiconic and tactile sensilla on the legs form two parallel somatotopic maps in the thoracic ganglia (Newland et al., 2000). Contrary to the well established tarsal taste system of flies (Murphey et al., 1989), mechanosensory and chemosensory axons from locust gustatory sensilla project to the same region of neuropil in the thoracic ganglion (Newland et al., 2000).

Vertebrate gustatory systems may also process chemical and mechanosensory information using overlapping somatotopic maps. The extra-oral taste system of catfish (*Ictalurus* sp.) is one example (Marui and Caprio, 1982; Hayama and Caprio, 1989). Catfish are crepuscular animals that live in turbid

waters. The skin of the catfish supports a gradient of taste receptors. More than 7 taste buds / mm^2 are found on the skin of the dorsum and belly and over 20 taste buds / mm^2 are found on the lips and the sensory barbles (whiskers) (Atema, 1971). Catfish use this extra-oral taste system to find and catch prey (Atema, 1971) and can discriminate over 28 different types of amino acids (Kanwal et al., 1987; Michel et al., 1993). Sensory afferents from extra-oral taste buds and tactile receptors on the skin project to a specialized region of the medulla oblongata called the facial lobe (Atema, 1971). Tactile and chemical afferents from the skin are organized somatotopically in the facial lobe, forming overlapping chemical and tactile projection maps of the body surface (Marui and Caprio, 1982; Hayama and Caprio, 1989).

Contact chemosensory systems of vertebrates that are neither gustatory nor olfactory are also organized somatotopically (Finger, 2000). The pectoral fin rays of the sea robin (*Prionotus carolinus*) are an excellent example (Finger, 1982; Finger, 1997; 2000). The fin rays of the sea robin are ventro-lateral extensions of the pectoral fin that support dense arrays of solitary chemoreceptor cells (Morrill, 1895, Whitear, 1971). These ventrally projecting chemosensory appendages are swept across the substrate and are used to find prey (Scharrer et al., 1947; Barbach and Case, 1965; Silver and Finger, 1984). Spinal nerves innervating each ray contain chemosensory, tactile, and proprioreceptive afferents that project to corresponding lobules in the dorsal horn of the spinal cord (Morrill, 1895; Herrick, 1907). These lobules are aligned somatotopically in a reversed anterior to posterior

order (Finger, 1982; Finger, 1997 for review). Ascending fibers from each lobule project to the cerebellum and optic tectum, suggesting that chemical and tactile information from the fin rays may be represented somatotopically in higher brain regions (Finger, 2000). With the potential exception of the scorpion pectine (Brownell, 1989; 1998) a similarly organized chemosensory appendage that is neither olfactory nor gustatory has not been described in arthropods.

We are just beginning to understand how chemosensory systems are organized in the second largest group of terrestrial arthropods, the arachnids (Foelix, 1985a; Sonenshine, 1991; Brownell, 1989, 1998; Strausfeld et al., 1998). This is due in part to a prevailing hypothesis at the turn of the century that chemical signaling was minimized in arachnids (Warburton, 1909) as evidenced by their lack of antennae (Brusca and Brusca, 1990). In the latter half of this century several studies have demonstrated that many arachnids have specialized chemosensory hairs, organs and appendages (Foelix and Axtell, 1972; Brownell and Farley, 1974; Ivanov and Balashov, 1979; Foelix, 1985a; Sonenshine, 1991; Gaffin and Brownell, 1997a; Brownell, 1998).

Muli-pourous olfactory hairs are located in special pits (the Haller's organ) on the first pair of walking legs of ticks (Arachnida: Acari) (Foelix, 1972; Hess and Vlimant, 1981; Sonenshine, 1991). Ticks use these olfactory organs to find potential hosts, mates and conspecifics (Schoni et al., 1984; Sonenshine, 1985; Norval, 1989; Yunker, 1990; Barre, 1998; McMahon and Guerin, 2000). Sensory neurons innervating sets of olfactory hairs both within and outside the Haller's

organ respond to CO_2, sulphides, methyl-salicylate, nitrophenols, and variety of other volatile odors from mammalian hosts and conspecifics (Wallade, 1982; Diehl et al., 1991; Steullet and Guerin, 1992a,b; Steullet and Guerin, 1994a,b). Putative "olfactory ganglia" are located in the fused synganglion of the tick, just medial to the pedal ganglia of the first pair of walking legs (Sonenshine, 1991; Szlendak and Oliver, 1992). The olfactory ganglia contain numerous glomeruli, but it remains to be determined if olfactory neurons from the Haller's organ project to this region (Sonenshine, 1991; Szlendak and Oliver, 1992).

The antenniform fore-legs of whip-scorpions (Arachnida: Uropygi) and sun-spiders (Arachnida: Solfuigdae) actively sample the environment and support cuticular hairs that resemble chemosensory and mechanosensory sensilla (Foelix, 1985a; Brownell, 1998). Sensory afferents from the forelegs of both arachnids project to a specialized glomerular neuropil in the cephalothoracic nerve mass (Brownell, 1998). Very little is known about the response properties and central projections of individual sensory neurons innervating these sensilla.

Numerous and diverse types of putative contact chemosensory sensilla are distributed on the appendages of spiders (Arachnida: Araneae) (Foelix, 1985a; Foelix, 1996). The physiology and central projections of these chemosensory sensilla have received little attention (Harris and Mill, 1979b; Foelix, 1985a; Anton and Barth; 1993; see Foelix, 1996, for review) even though olfactory and substrate borne chemical cues mediate intraspecific communication in many spiders (Hegdekar and Dondale, 1969; Suter and Renkes, 1982; Schultz and Toft, 1993;

Trabalon et al., 1997). Axons from chemosensory hairs on the legs of *Cupiennius salei* appear to project somatotopically into one of the ventral tracts of the SOM (Anton and Barth, 1993). It is unclear if mechanosensory and chemosensory axons from individual chemosensory hairs are anatomically segregated in the nervous system of arachnids.

The pectines of scorpions (Arachnida: Scorpiones) are contact chemosensory appendages that cannot be classified as olfactory or gustatory organs. The peg sensilla have a single apical pore, resembling contact chemosensory sensilla of other arachnids and insects (Slifer, 1970; Foelix, 1985a). Sensory neurons innervating the peg sensilla respond to a diverse range of chemicals, including ketones, alcohols, and aldehydes (Gaffin and Brownell, 1997a) but do not respond to classic gustatory stimuli (Hoffman, 1967). Behavioral studies also indicate that the pectines are not directly involved in gustation (Root, 1990). Prey items are captured by the pedipalps and macerated by the chelicerae. The chelicerae support sets of chemosensory hairs that respond to classic gustatory stimuli (Venkateswara, 1963; Root, 1990), suggesting that these sensilla may mediate appetitive behaviors. The pectines are also not necessary for the detection of substrate moisture (Gaffin et al., 1992), although sensory neurons innervating the peg sensilla respond to humid stimuli (Gaffin and Brownell, 1997a). Imbibitory behaviors of desert scorpions are only altered when the tarsi are selectively blocked with wax, suggesting that chemosensory hairs on the tarsus of scorpions detect substrate moisture (Gaffin et al., 1992).

As previously stated, the pectines of scorpions are chemosensory appendages that appear to share certain organizational features with sensory systems that encode properties of physical space (e.g. visual and somatosensory systems). The pectines support a two-dimensional array of uniform sensilla that are swept across the substrate (Swoveland, 1976; Gaffin and Brownell, 1992). Sensory neurons innervating the peg sensilla form a plexus of peripheral axo-axonic synapses (Foelix and Muller-Vorholt, 1983) and sensory axons from the pectinal teeth are arranged in topographic register in the pectinal neuropil, forming an internal 'map' of this sensory array (Brownell, 1989; 1998). Peripheral processing and central topography in this sensory system may have functional significance related to high resolution 'imaging' of both substrate texture and chemistry.

Forel (1929) proposed that ants could use a similar "topochemical" sense to follow chemical trails. Ants use a variety of substrate deposited chemicals to orient and recruit nest-mates to food sources and potential threats (Holldobler and Wilson, 1970, 1990; Vander Meer et al., 1990; Traniello and Robson, 1995 review). These secretions are applied periodically by 'dotting' or continuously 'dragging' a gland (e.g. Dufour's gland) across the substrate. This creates a trail of chemical s that nest mates follow (Wilson, 1959; Holldobler and Traniello, 1980; Traniello, 1980). Forel (1929) proposed that the spatial pattern of chemical signals deposited on the substrate could encode information about food quality and location (see Wilson and Bossert, 1963).

Wilson and Bossert (1963) rejected Forel's (1929) "topochemical" sense because "although human can make out shapes visually, the ants cannot do so by chemoreception"(Wilson and Bossert, 1963) and provided a much simpler behavioral trailing mechanism. Deposited pheromones on a trail could diffuse into the air, forming a localized active space where the chemical concentration would be high (Wilson and Bossert, 1963). Behavioral studies by Hangartner (1967) support this hypothesis. Ants follow chemical trails using their paired antennae. Olfactory hairs on the antennae detect pheromone concentration and the boundary of the odor space is determined as the antennae are swept from side to side (Hangartner 1967; Traniello and Robson, 1995 for review). This allows for a bilateral comparison of pheromone concentration across the substrate that does not require the fine spatial resolution of a chemical signal (Dusenberry, 1993 for review).

The role of the pectines in chemically mediated behaviors will have to be determined before we can test Forel's (1926) hypothesis in scorpions. Studies that investigate mate-trailing in scorpions could serve as an important first step in this process. Behavioral studies have demonstrated that the pectines are used to choose suitable substrates for burrowing and spermatophore deposition (Alexander, 1957; Alexander, 1959; Abushama, 1964) and may be used to sense substrate borne chemical cues, including sex pheromones (Krapf, 1986; Gaffin and Brownell, 1992). Scorpions could also trail mates, conspecifics, and prey using a behavioral mechanism that does not require the fine spatial resolution chemical signals. The

functional neuroanatomy of the scorpion pectine should be considered suggestive until further behavioral studies have been conducted.

An alternative hypothesis is that peripheral processing of chemosensory stimuli is an ancestral characteristic of the scorpion nervous system. Peripheral synapses are a common feature of arachnid and xiphosuran sensory systems (Foelix, 1985b; Farley, 1999; Fahrenbach, 1999). The whip-like forelegs of whip-spiders (Arachnida: Amblpygi) support several thousand tactile and chemosensory hairs. Sensory afferents from the tactile hairs form *en-passant* synapses on the dendrites, somata, and axons of peripheral giant interneurons within the whips (Foelix, 1975; Foelix and Troyer, 1980; Foelix, 1985b). These excitatory synapses mediate rapid withdrawal of the forelegs when the tip of the whip is touched (Igelmund and Wendler, 1991a,b). Peripheral synapses are also found on the axons, somata and or dendrites of sensory neurons innervating tactile hairs, lyriform organs, and internal joint receptors of spiders (Arachnida: Araneae) (Foelix and Choms, 1979; Foelix, 1985b; Fabian-Fine et al., 1999a,b).

Synaptic plexi are found directly beneath the compound eyes of the horshoecrab (*Limulus*) (Fahrenbach, 1999 for review). Axon collaterals from the eccentric cells innervating each ommatidium form a synaptic plexus involved in lateral inhibition (Ratliff et al., 1966; Hartline and Ratliff, 1974; Fahrenbach, 1985). Peripheral synapses from axon collaterals are also associated with arrays of chemosensory sensilla on the flabellum, chilaria, and endopodites of the book gill covers (Hayes, 1971; Griffin and Fahrenbach, 1977; Hayes and Barber, 1982; see

Fahrenbach, 1999 for review). The ancestral position of scorpions and merostomates in the sub-phylum Chelicerata (Stormer, 1969; Brusca and Brusca, 1990; Dunlop and Webster, 1999) suggests that peripheral synaptic plexi may be plesiomorphic character trait of arachnid sensory systems.

Chapter 2

Cytoarchitecture of Primary Sensory Neurons in the Pectines of Scorpions

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Abstract

The primary chemosensory organs of scorpions (Arachnida: Scorpionida) are the pectines, paired ventral appendages that intermittently brush the substrate as the animal walks. Each pectine supports thousands of chemoreceptive sensilla, called 'pegs', arranged in two-dimensional arrays on the ventral surface of numerous teeth that make up this comb-like organ. We used confocal laser scanning, light and transmission electron microscopy to describe the 3-dimensional organization of sensory neurons within the pectinal teeth of the sand scorpion, *Paruroctonus mesaensis*. Histologically, each pectinal tooth contained a layer of sensory dendrites, somata, and axons. The layer of sensory somata was further divided into an outer nuclear layer, containing numerous small bipolar cells (3.6 μm mean diameter) identified as chemoreceptors by the termination of their dendrites near the pore opening at the tip of the peg sensillum, and an inner nuclear layer of large bipolar neurons (5.6 μm mean diameter) identified as mechanoreceptors by the termination of their dendrites at the base of each peg sensillum. The axonal lamina was immunoreactive to antibodies against the synaptic vesicle protein Synapsin, resembling the synaptic plexiform layer described in ultrastructural studies of the pectinal teeth. Each peg sensillum was innervated by a cassette of neurons that gave rise to axonal swellings characteristic of *en-passant* synapses within the axonal laminae. The scorpion pectine is unique among invertebrate chemosensory systems in peripheral organization. Dense two-

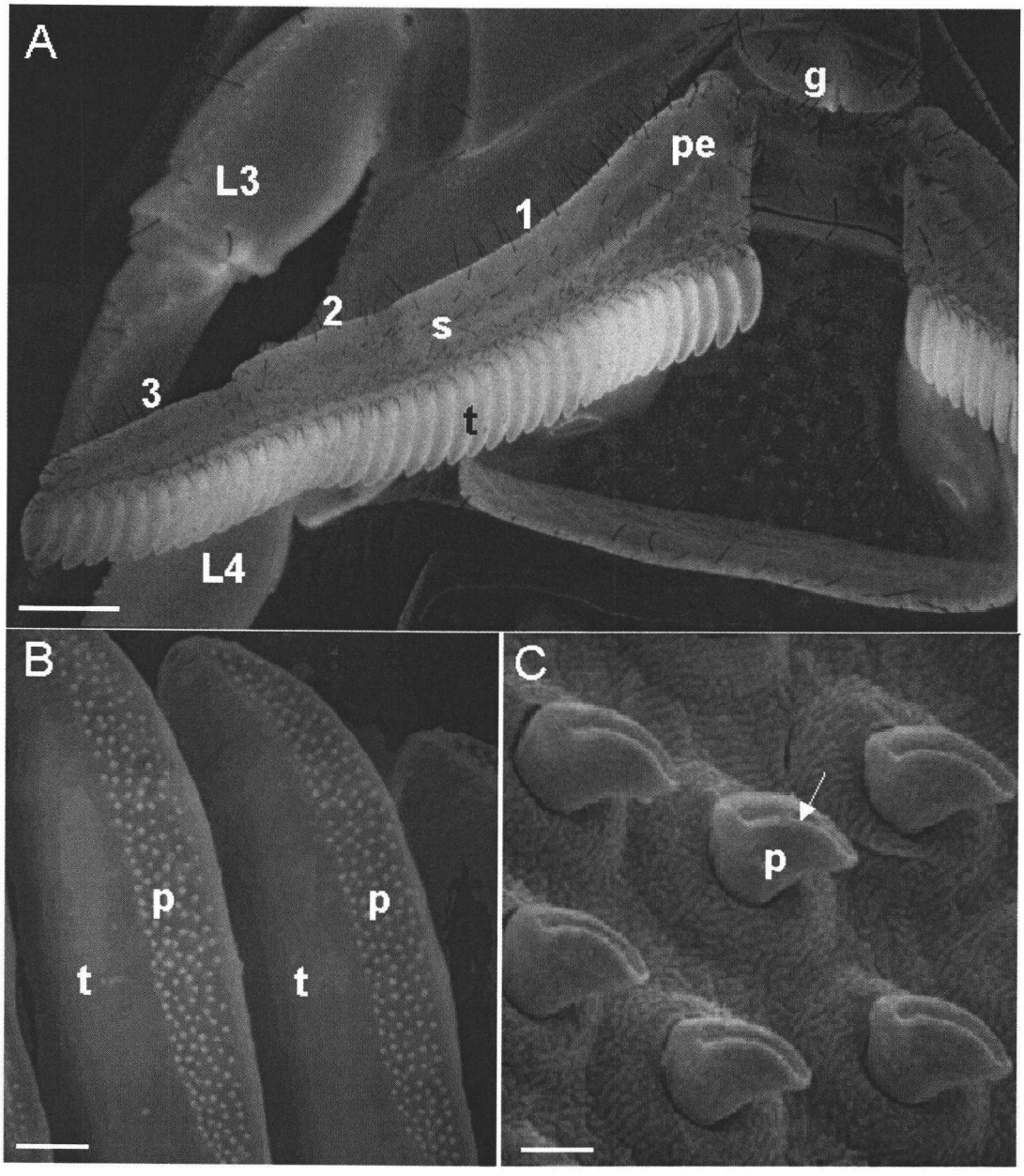
dimensional fields of sensilla process chemical and mechanical information through spatially arranged cassettes of cells before transmission to the central nervous, suggesting that features of substrate texture and chemistry can be detected with topographical precision.

Introduction

This is a neuroanatomical study of a specialized chemosensory appendage found only in scorpions. The pectines are paired ventral appendages originating from the second mesosomal segment (somite XI) located just posterior to the fourth pair of walking legs (Hjelle, 1990) and genital aperture. A distinct attribute of this chemosensory organ is the dense two-dimensional field of sensilla (15,000-25,000 sensilla / mm^2) it supports (Brownell, pers obs). In the desert sand scorpion (*Paruroctonus mesaensis*) each pectine consists of a jointed spine from which 30 to 40 slipper shaped teeth extend in a comb-like arrangement (Fig. 2.1a). Each tooth supports several hundred short (< 5 μm) setaform sensilla called pegs, which are arranged in a flat array (Fig. 2.1b-c). By ultrastructure, the pegs resemble chemosensory hairs found in other arthropods (Slifer, 1970; Foelix, 1985a for review). Each peg sensillum has a slit shaped terminal pore that is innervated by 9-15 chemosensory dendrites. At the base of each peg, a single dendrite terminates in a tubular body characteristic of mechanoreceptors in other setaform sensilla (Ivanov and Balashov, 1979; Foelix and Muller-Vorholt, 1983; Foelix, 1985a).

The biological functions of the pectines are uncertain, although they are sexually dimorphic and actively sweep the surface during courtship, allowing close apposition of the peg sensilla to the substrate (Polis and Farley, 1979; Gaffin and Brownell, 1992). Behavioral studies (Alexander, 1957; Alexander, 1959; Abushama, 1964; Krapf, 1986) support Schroder's hypothesis (1908) that the

Figure 2.1 Morphology of the pectinal teeth.
(A) Ventral view of the cephalothorax of the desert sand scorpion (*P. mesaensis*) showing the right pectine and 38 teeth (t). The pectinal appendages (pe) articulate with the cephalothorax just posterior to the genital operculum (g) and are swept over the substrate beneath the third (L3) and fourth (L4) pair of walking legs. Each pectine has a basal spine (s) composed of three (1-3) segments that support the numerous sensilla bearing teeth (t). (B) Scanning electron micrograph of the ventral surface of two pectinal teeth (t) showing the flat field of peg sensilla on each tooth (p). (C) Scanning electron micrograph showing several spade-shaped peg sensilla (p). The slit-like terminal pore is visible along the top edge of the sensillum (arrow). Scale A = 1 mm, B = 50 μm, C = 3 μm.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pectines detect both mechanical and chemical stimuli and probably mediate sensitivity to surface textures and substrate borne chemical cues, including sex pheromones (Gaffin and Brownell, 1992). Electrophysiological recordings from the peg sensilla confirm that the sensilla respond to both mechanical and chemical modalities (Hoffman, 1964; Gaffin and Brownell, 1997a).

The pectines are unique among invertebrate chemosensory systems in that they share organizational features with sensory systems that encode properties of physical space, such as visual and somatosensory systems. In addition to the small size and high density of uniform sensory elements (sensilla), sensory neurons innervating the peg sensilla form a plexus of peripheral axo-axonic synapses (Foelix and Muller-Vorholt, 1983) that may be the sites of synaptic excitation and inhibition known to occur between primary sensory cells in each sensillum (Gaffin and Brownell, 1997b). Sensory axons from the pectinal teeth project to a discrete neuropile at the caudal end of the cephalothoracic nerve mass, where they are arranged in topographic register as an internal 'map' of the sensory array (Brownell, 1989; 1998). Peripheral processing and central topography in the pectinal sensory system may have functional significance related to high resolution 'imaging' of both substrate texture and chemistry.

The cytoarchitecture of sensory neurons within this specialized appendage has not been described. This is surprising given that Gaskell (1902) and Schroder (1908) first investigated the histology of the pectinal teeth almost 100 years ago. In the latter half of this century, several studies have focused on the ultrastructure of

the peg sensilla (Carthy, 1966; Carthy, 1968; Ivanov and Balashov, 1979; Foelix and Muller-Vorholt, 1983) and the functional anatomy of the central nervous system (Brownell, 1989; Brownell, 1998). The objective of the present study was to use different and complimentary anatomical approaches to describe the organization of sensory neurons within the pectinal teeth of the sand scorpion *P. mesaensis*, and to investigate the location and extent of peripheral synaptic plexi. This was accomplished using standard immunological, histological and neuroanatomical methods coupled with confocal laser scanning, light and transmission electron microscopy.

Materials and Methods

Animals

Adult male and female sand scorpions (*P. mesaensis*) used in this study were collected in the Mojave Desert using portable blacklights (Stahnke, 1972). The animals were housed in plastic 12 oz containers with 20 ml of sand, conditioned to phase-shifted light cycles (nightfall occurring at 4:30 PM) and daily heat cycles of 28-35°C to approximate natural dune environments in the east Mojave Desert (Brownell, unpublished data). The animals were maintained on a diet of one wax worm and a few drops of water given once every three weeks.

Transmission Electron Microscopy

The pectines from male and female sand scorpions were removed and cut into segments of 1-3 teeth and placed in fixative containing 0.1 M cacodylate buffer, 2% paraformaldehyde, 2.5% glutaraldehyde, 1% sucrose and 0.16 M $CaCl_2$ for 24 hrs at 4°C. Tissues were then post-fixed in OsO_4 for 2-4 hrs, dehydrated in serial ethanol dilutions and embedded in hard Spurs resin. Thin sections (1-5 μm) were stained with 1% Methylene Blue / Azure II (solution #1 from Humphrey and Pittman, 1974) for histological observation. Ultrathin sections were mounted on copper grids coated with Butvar (Ted Pella Inc.) and contrast enhanced using an abbreviated lead citrate, uranyl acetate method (Daddow, 1986). Grids were viewed on a Phillips CM-12 scanning transmission electron microscope (STEM).

Histology

Rapid collonier Golgi techniques commonly used in insects (Strausfeld, 1980) were adjusted for use in scorpions. The pectines were removed and placed in physiological saline and minimally dissected into individual pectinal teeth. Tissues were pre-fixed in 2.5% glutaraldehyde in Millonigs buffer with 3.5% sucrose for 4-5 hrs. Tissues were washed in buffer, incubated in 2.5% potassium dichromate with 3.5% sucrose, placed in a mixture containing 2.5% potassium dichromate (with sucrose) and 25% glutaraldehyde (5:1 ratio) and then stored at 4°C for 5 days in a dark chamber. Pectinal teeth were washed in cold potassium dichromate (no sucrose) and decanted into a solution of 1% OsO_4 and 2.5% potassium dichromate (no sucrose) (1:100 ratio) and stored at 4°C for 5 days in a dark chamber. Pectinal teeth were then washed (3x10 min) in distilled water and incubated in 0.75% $AgNO_3$ at 4°C for 48 hrs in a dark chamber. Golgi preparations were dehydrated in alcohol, cleared in methyl salicylate, infiltrated with Durkupan plastic and mounted between glass coverslips for observation using compound light microscopy and reflectance confocal microscopy. Successful Golgi impregnations were drawn at 400-1000x using a Leitz compound light microscope mounted with a camera-lucida or photographed on a Leitz DMRB compound light microscope using Ektachrome 400 Elite II film.

Procedures for retrograde filling of insect nerves (Mesce et al., 1993) were adapted for use in scorpions. Male and female sand scorpions (*P. mesaensis*) were

immobilized ventral side up on glass slides using plasticine clay such that the pectines could be manipulated and positioned on coverslips using double-sided adhesive tape. A small well of petroleum jelly was formed at the base of the pectine and filled with scorpion saline. The pectinal nerve was cut close to the base of the pectine where it articulates with the mesosoma and then isolated in the well. To promote axonal swelling the well was filled with distilled water for 30 seconds, then replaced with scorpion saline and a few crystals of Neurobiotin (Vector Labs). The pectinal nerve was sealed in the well for 10-14 hrs at room temperature. The pectines were then cut from the animal, dissected into individual teeth in scorpion saline and fixed in 4% paraformaldehyde and 0.16% glutaraldehyde in 0.2 M PBS for 2-6 hrs. Fixed tissues were washed in 0.2 M PBS, permeabilized in 3% Triton-X-100 in 0.2 M PBS for 1 hr, and then incubated in a 200/1 mixture of 0.2 M PBS and strepavidin conjugated to indocarbocyanine (Cy3 from Jackson Immuno Research Labs) for 24-48 hrs, followed by another incubation in a 2000/1 mixture of 0.2 M PBS and 4', 6-diamidino-2-phenylindole, dihydrochloride (DAPI from Molecular Probes Inc.) to label cell nuclei. Pectinal teeth were dehydrated in serial alcohol dilutions, cleared in methyl salicylate and mounted in Cytoseal (Sigma inc.) for confocal observation.

Immunological Methods

Immunohistochemical staining techniques were adapted from procedures used successfully on the peripheral synapses of spiders (Fabian-Fine et al., 1999a,b). Freshly dissected pectines were placed in 0.1 M PBS containing 4% paraformaldehyde and individual teeth were dissected free with tips cut open to increase antibody penetration. After 1-2 hrs in fixative the tissues were washed in 0.1 M PBS (3x15 min) permeabilized with 3% Triton-X-100 in 0.1 M PBS (3x30 min) and then pre-incubated in blocking medium (0.1 M PBS with 5% nonfat milk powder and 0.1% TX-100) for 30 min followed by incubation for 48 hrs at 4°C in blocking medium containing a 1:100 dilution of SYNORF1, a monoclonal antibody raised against *Drosophila*-synapsin (Klaggs et al., 1996). The pectinal teeth were then rinsed in 0.1 M PBS (3x 15 min) and incubated for 48 hrs at 4°C in blocking medium containing a 1:500 dilution of goat anti-mouse IgG coupled to Cy3 (Jackson Immuno Research Labs) and were then rinsed in 0.1 M PBS (3x 30 min) containing a 1:500 dilution of DAPI (Molecular probes) to label cell nuclei. The pectinal teeth were then dehydrated and mounted for confocal observation as described above.

Anti-synapsin binding specificity of scorpion tissue was tested using SDS-PAGE gel electrophoresis with immuno-blotting. Scorpions were anesthetized using CO_2 and the pectines and cephalothoracic nerve mass were removed and placed in 70% ethanol. The pectines were then dissected leaving only the pectinal

teeth. Pectinal teeth and central nerve masses were placed in separate 1 ml Eppendorf tubes and freeze-dried. Each sample was then solubilized in 20 µl of sample buffer (1% sodiumdodecylsulfate, 0.025 M Tris-HCl, pH 6.8, 0.001% bromophenol blue, 0.192 M glycine). Samples were boiled in buffer for 5 min followed by electrophoresis and gel transfer to a nitrocellulose membrane. Blots were treated with SYNORF1 (1:500 dilution) in 0.1 M PBS with 0.05% Tween-20 at 4°C for 1 hour, and then incubated in goat anti-mouse IgG coupled to horseradish peroxidase (Jackson Immuno Research Labs) diluted 1:50,000. Western blots were enhanced using a chemiluminescence system (SuperSignal Substrate, Pierce Inc.) and developed on film.

Confocal Microscopy

Scorpion epicuticle is auto-fluorescent under ultraviolet illumination allowing cuticular structures to be visualized with selectively labeled neuronal tissues. Confocal images were captured with a Leitz DM IRBE inverted confocal scanning laser microscope (CSLM) using Leica True Confocal Scanning 4D Software. Fluorescent channels were scanned separately and line averaged 8-16 times to maximize resolution. The following laser lines (L) and Leica emission filters were used to visualize scorpion epicuticle and the fluorescent dyes Cy3, and DAPI respectively: L 488λ and a band pass 520λ filter, L 588 and a long pass 590λ filter, L 351 and a band pass 440λ filter. Maximum projection images were constructed using Leica True Confocal Scanning 4D Software. Two and three

channel images (cuticle, Cy3, and DAPI) were constructed using Adobe PhotoShop (version 5). Maximum projection images were converted to R.G.B. format and overlaid using the *Apply-image* function. Histological measurements were made using Scion Image software (version 3B).

Results

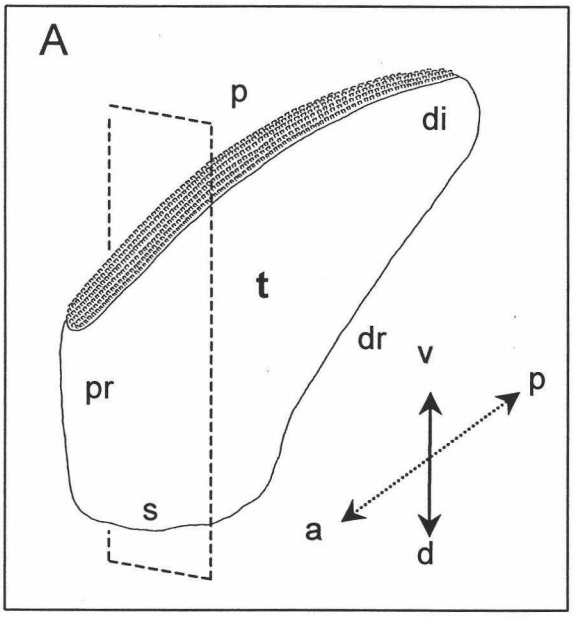
External Morphology of the Pectinal Teeth

Adult male sand scorpions of medium size (carapace length < 6 mm) were used in this study. The pectines from animals in this size class have 35 teeth on average (mean = 35.14, $\pm$1.9, n = 20) with peg fields from the medial teeth (pectinal teeth 15-17) supporting a modest density of 200-300 peg sensilla per tooth (mean = 239, $\pm$41.9, n = 4). The pectinal teeth of large male *P. mesaensis* (carapace length > 8 mm) can support over 1500 peg sensilla (Swoveland, 1978). The external morphological characters of the pectinal teeth serve as reference points for our observations (Fig 2.2a).

Histology and Organization of Sensory Afferents

The pectinal teeth were divided horizontally into three layers (Fig. 2.2b). Just underneath (10 μm) the field of peg sensilla was a 30 μm thick layer of accessory cells followed by a 100-120 μm thick layer of neuronal somata. These two layers of cells filled the majority of space within the pectinal tooth. Directly below the layer of neuronal somata was a layer of axonal processes running parallel to the dorsal ridge of the pectinal tooth. This axonal lamina tapered anteriorally to form a single nerve within the stalk of the tooth and then joined the large main nerve within the pectinal spine.

Figure 2.2 External morphology and cytoarchitecture of a single pectinal tooth. (A) Each tooth (t) is a slipper-shaped structure with a ventral surface that supports the field of peg sensilla (p). The proximal margin (pr) and distal tip (di) of the tooth are located at the anterior and posterior ventral edges of the sensillar field. The distal tip projects dorsally and anteriorally forming a dorsal ridge (dr) of cuticle that joins with the supportive stalk (st). The stalk (s) forms a flexible joint of attachment with the pectinal spine. Arrows indicate the dorsal-ventral and anterior-posterior axis (a = anterior, p = posterior, d = dorsal, v = ventral). Dashed line = location of cross section for panel B. (B) Mid-longitudinal cross section of a pectinal tooth stained with methylene blue – azure II. Three histological layers are evident. Below the peg sensilla (p) is a diverse layer of accessory cells (ac) and a layer of neuronal cells (nc). A layer of axons forming the pectinal tooth nerve (nv) is visible in the stalk of the tooth. Scale = 10 μm.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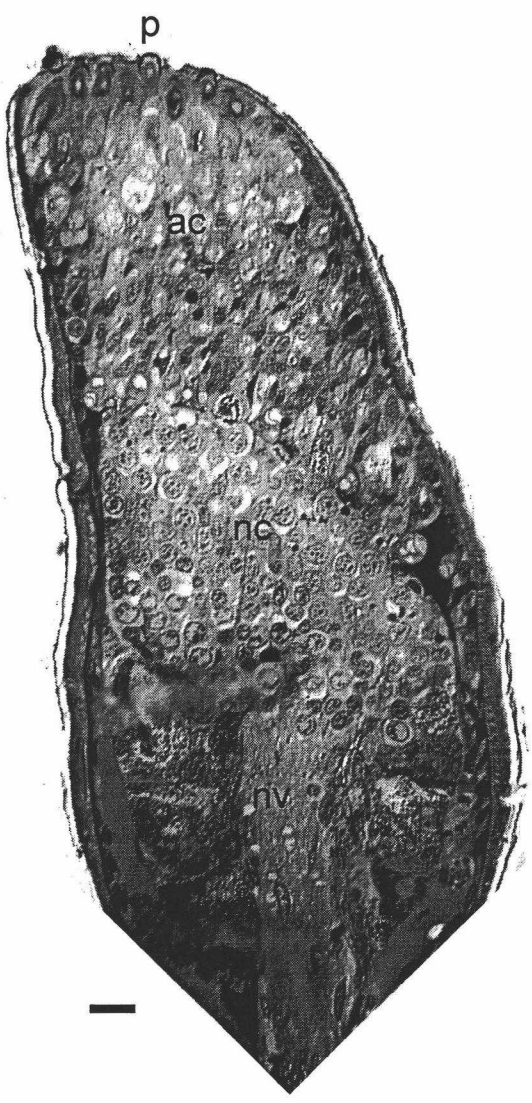
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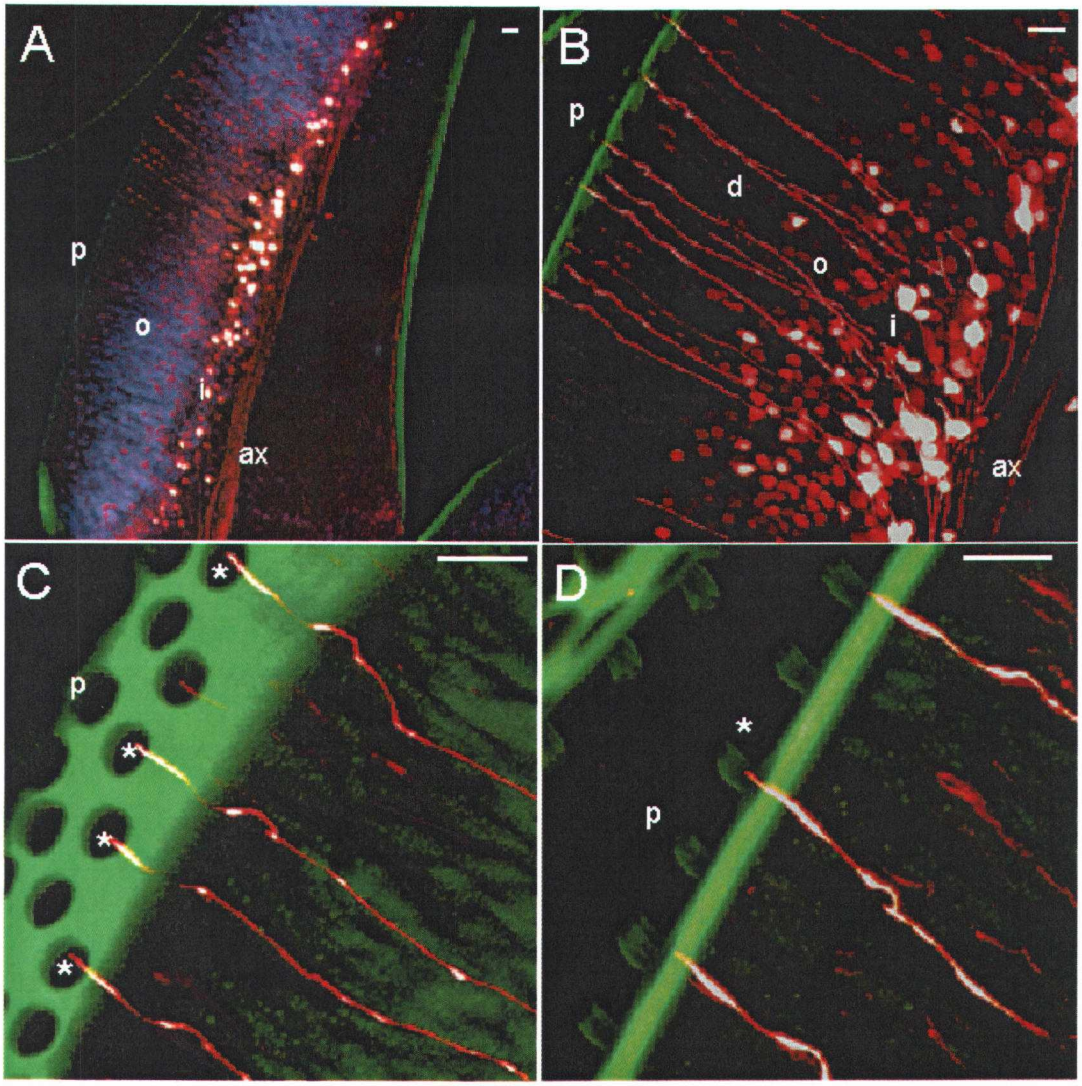
Sensory afferents within the pectinal teeth were organized orthogonal to the field of peg sensilla, giving the appearance that each tooth was composed of distinct laminae of neuronal elements (Fig. 2.3a). Dendrites innervating the peg sensilla originated from two layers of somata in the neuronal cell layer. The first layer of somata was 75-90 μm thick and was composed of small spherical bipolar sensory neurons (mean diameter = 3.6 μm). The second layer of somata was located 100-130 μm below the sensillar field and consisted of a thin strip of tissue (30-35 μm) filled with larger bipolar neurons (mean diameter = 5.56 μm) (Fig. 2.3b). We compared the mean diameter of somata in each nuclear layer and found that they were significantly different (Students t-stat = 7.852, $P > 0.001$, n = 25 for outer and n = 29 for inner lamina). Hereafter, we will refer to the distal lamina as the outer nuclear layer (ONL) and the proximal lamina as the inner nuclear layer (INL). Sensory neurons in each layer innervated the peg sensilla differently: dendrites from cells in the INL terminated at the base of the peg sensilla and dendrites from neurons in the ONL projected through the sensillar shaft of the peg sensilla, terminating near the sensillar pore (Fig. 2.3c-d). Below the INL was a plate of axonal fibers (20-25 μm wide) that encompassed the full length and width of the dorsal ridge of the pectinal tooth. Numerous axonal swellings were observed in this axonal layer (Fig. 2.4). This plate of axons formed a single nerve (30-35 μm wide and 80-100 μm long) near the proximal margin of the tooth and projected into the pectinal spine to join the main pectinal nerve.

Figure 2.3 Laminar organization of sensory afferents within a pectinal tooth. (A) Confocal image of a pectinal tooth showing labeled sensory axons (ax) and inner (i) and outer (o) layers of cell bodies from sensory neurons innervating the peg sensilla (p). The fields of peg sensilla are located on the left side of each panel. (B) Enlarged view of panel A showing the difference in cell diameter and layer width of the inner (i) and outer (o) nuclear layers. Dendrites (d) originating from sensory neurons in both layers innervate the peg sensilla. (C) Confocal image of an array of parallel dendrites terminating at the base (*) of individual sensilla. (D) Confocal image of a labeled dendrite inside the shaft (*) of a peg sensillum. Three sets of optical sections (one for each color) were superimposed to create the image in Panel A. Each maximum projection image consisted of 25 optical sections through 54 μm of tissue. Panels B-D are 2 channel maximum projection images 17 μm deep composed of 20 optical sections. For all panels green = cuticle, red = labeled afferents, blue = DAPI stained nuclei. Scale A-D = 10 μm.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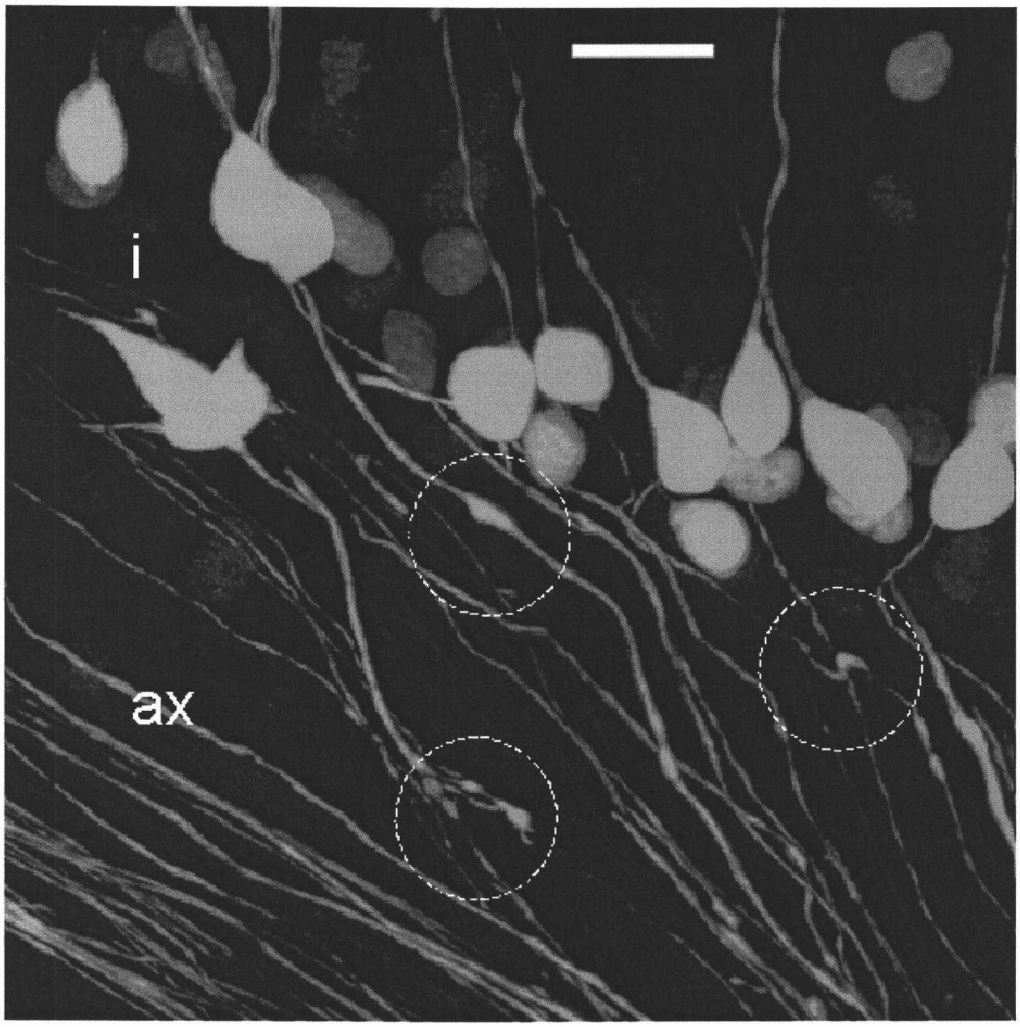

Figure 2.4 Axonic swellings and axo-axonic contacts between sensory afferents. In this confocal image many axons from labeled sensory neurons in the inner nuclear layer (i) can be seen forming complex axo-axonic contacts (circles) with other sensory axons as they project into the axonal lamina (ax). This is a maximum projection image composed of 25 optical sections spaced at 1μm intervals. Scale = 10 μm.

Synapsin-like Immunoreactivity

Pectinal teeth treated with the monoclonal antibody SYNORF1 contained immunolabeling in the pectinal tooth nerve and axonal lamina (Fig. 2.5a). Many immunofluorescent blebs 0.8-2.0 μm in diameter (mean = 1.11 μm, $\pm$0.37 μm, n = 53) were located in the axonal lamina. Specific immunofluorescence was not observed when the pectinal teeth were treated with only secondary antibody (Fig. 2.5b). Western blots from CNS and pectinal teeth preparations showed matching bands in all immuno-blot trials (n = 4) with dominant bands at 70 kDa and smaller bands at 60 and 40 kDa (Fig. 2.5c).

Innervation of the peg sensilla

The peg sensilla appear to be innervated by a regular number of dendrites arranged in a specific pattern forming a cartridge or cassette of cells. Ten to fifteen dendrites innervated each peg sensillum (mean = 11 dendrites, $\pm$1.76, n = 10) with most of these terminating near the sensillar pore within the sensillar shaft (Fig. 2.6a). A single microvillar cell enveloped each cassette of dendrites as they innervated a peg sensillum. At the level of the inner dendritic segments the cassette of dendrites showed an axis of polarity relative to the microvillar cell (Fig. 2.6b). Small dendrites (4-10) in each cassette were found nearest the microvillar cell forming one pole while a set of 3-5 larger dendrites were located distally, forming the opposite pole (Fig. 2.6c). The dendrites innervating the peg sensilla originated

from numerous somata located in the neuronal cell layer in the pectinal tooth (Fig. 2.6d). The mean number of inner and outer dendritic segments innervating a peg sensillum was not statistically different (mean number of dendritic inner segments = 11, $\pm$1.76, n = 10, mean number of dendritic outer segments = 12.2, $\pm$1.76, n = 10, Students t-stat = -1.58, P>0.1) suggesting that dendrites do not branch as they project into the peg sensilla.

Cytoarchitecture of the Sensillar Cassette

The peg sensilla were innervated by parallel cassettes of sensory neurons comprised of the two types of neurons observed in the outer and inner nuclear layers (Fig. 2.7a-b). The majority of fibers innervating the peg sensilla originated from the numerous small cells in the outer nuclear layer. Large aggregations of these neurons were often stained within a single cassette obscuring their morphology. In a few cases, bipolar nuclei within the cassette could be discerned (Fig. 2.7c). Three to five larger bipolar neurons with cell bodies in the inner nuclear layer also innervated each peg sensillum. The larger neurons were located below the smaller neurons (Fig. 2.7c) and were stained singularly or in small groups allowing the sensory axons and dendrites to be clearly distinguished (Fig. 2.7d).

Axonal Varicosities

Sensory axons in silver impregnated tissues showed numerous swellings and apparent contacts with other sensory axons and somata. These axonal swellings were 1-1.5 μm in diameter and were classified into the following three types. In a few cases, the axons of sensory neurons innervating neighboring sensilla formed axo-axonic appositions as they entered the axonal lamina and pectinal 'tooth' nerve (Fig. 2.8a-b). In contrast, the majority of axonal swellings observed were between axons of sensory neurons innervating the same peg sensilla (Fig. 2.8c). Axo-somatic contacts were also observed but were uncommon (Fig. 2.8d). Numerous axonal varicosities were located in the pectinal tooth nerve.

Figure 2.5 Synapsin-like immunoreactivity within a pectinal tooth.
(A) Whole mount preparation of a pectinal tooth treated with SYNORF1, a monoclonal antibody that binds to presynaptic terminals. Specific immunolabeling is restricted to axons in the pectinal tooth nerve (nv) and the axonal lamina (ax) below the two nuclear layers (nc). (B) Whole mount preparation of a pectinal tooth treated with only the fluorescent secondary antibody. Specific immunolabeling is not observed within the pectinal teeth. (C) Synapsin-like immunoreactivity of scorpion brain and pectinal teeth (*P. mesaensis)* revealed by Western Blot analysis. Matching bands are visible in samples from scorpion brain (b) and pectinal teeth (t). A dominant band is located at 70 kDa with smaller matching bands visible near 60 and 40 kDa. Panels A and B are maximum projection images consisting of 20 optical sections through 53 μm of tissue (Panel A) and 16 optical sections through 32 μm of tissue (Panel B). ac-d = accessory cell- dendritic layer, dr = dorsal ridge of the tooth, p = field of peg sensilla, pr = proximal margin, t = pectinal tooth. Scale A = 33 μm, B = 100 μm

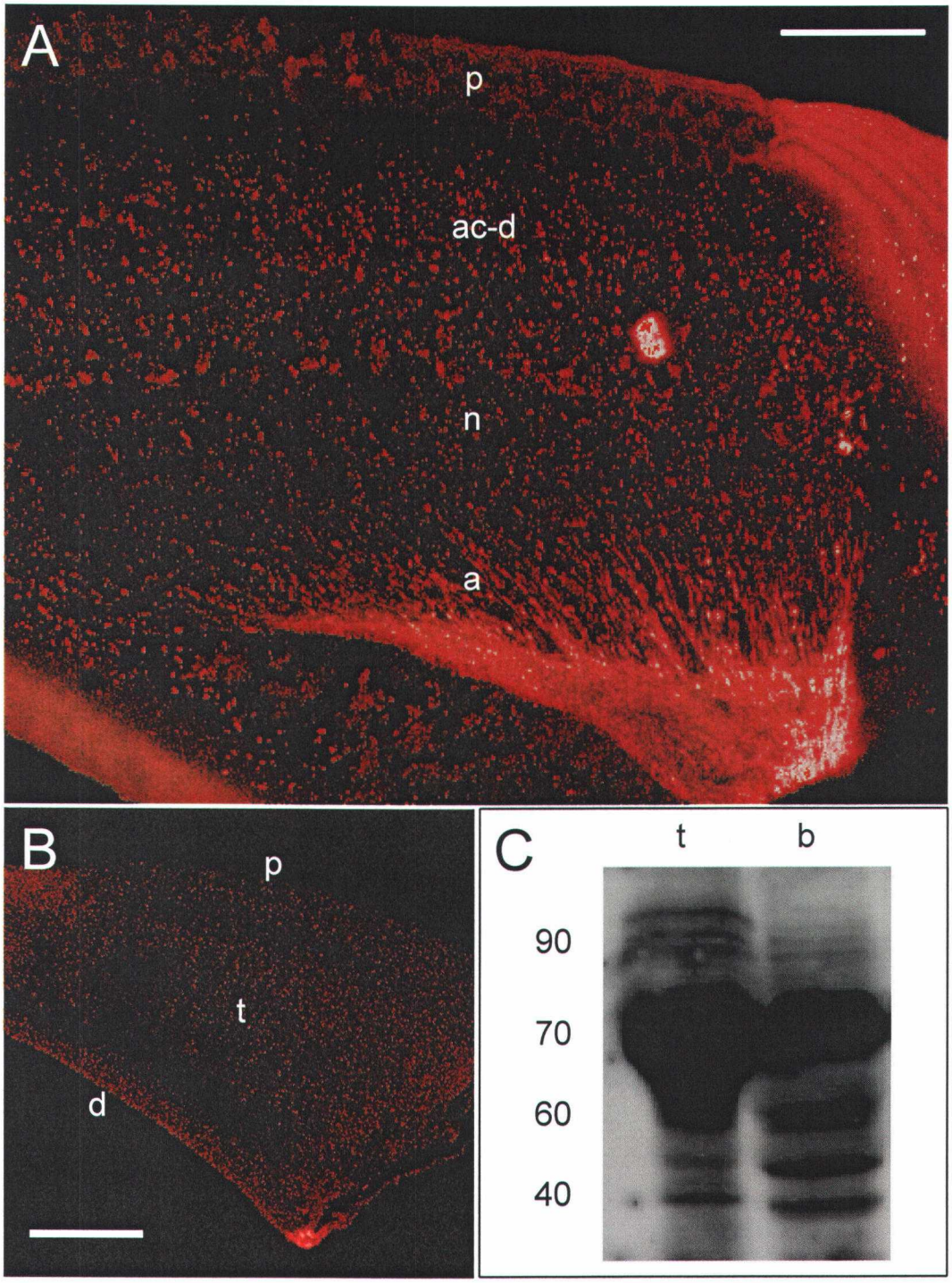
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Figure 2.6 Innervation of a peg sensilla revealed by serial ultra-thin sections. (A) Cross-section through the shaft of a peg sensillum showing the outer cuticle (c) of the sensillum and a dendritic sheath (ds) surrounding several dendrites (d). (B) Cross-section through the cassette of sensory dendrites innervating the peg sensillum at the level of the outer segments. The dendrites (box) are all ensheathed by a single microvillar cell (cm). (C) Cross-section through a sensillar cassette at the level of the inner segments. Many smaller dendrites (*) are located next to the microvillar cell. A set of larger dendrites (1-3) can be seen opposite the microvillar cell. (D) A medial cross-section through a pectinal tooth showing neuronal somata. Scale A-D = 1 μm.

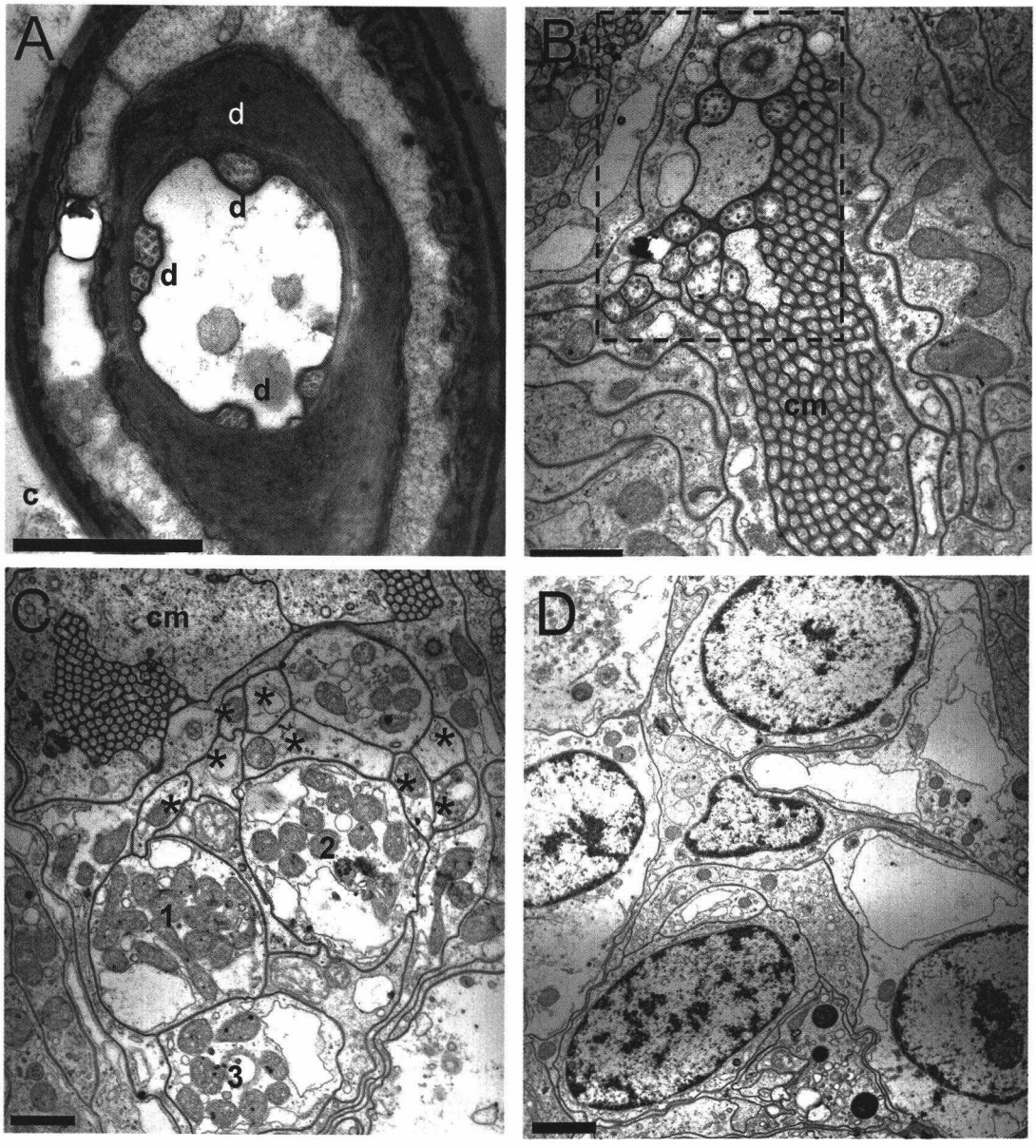
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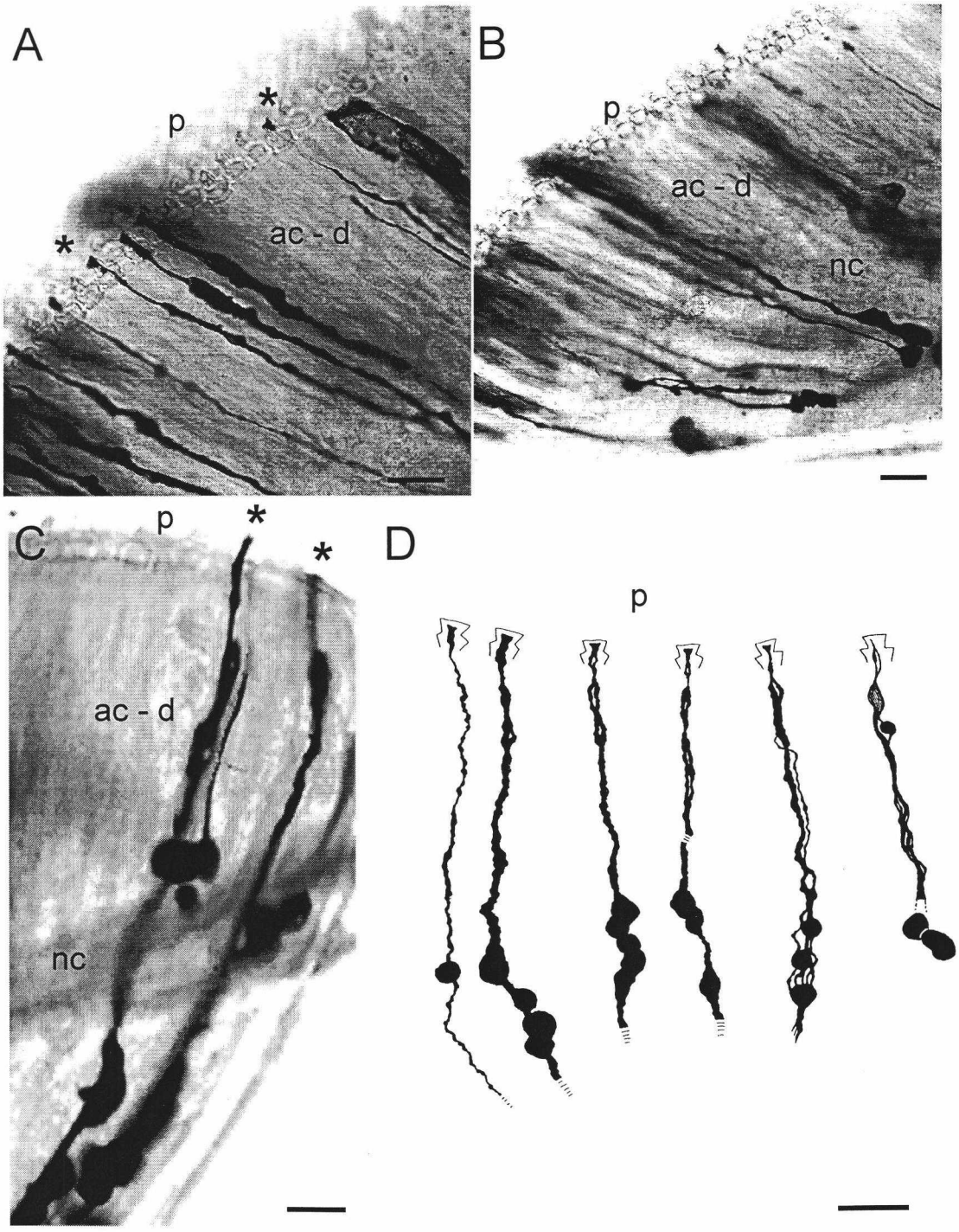
Figure 2.7 Cellular composition of the sensillar cassette.
(A) Confocal reflectance image of Golgi impregnated dendrites (*) projecting through the accessory cell – dendritic layer (ac-d) to the peg sensilla (p). (B) Confocal reflectance image of Golgi impregnated somata in the neuronal cell layer (nc) from dendrites in panel A. (C) Compound micrograph showing cassettes of dendrites (*) innervating two peg sensilla. The dendrites originate from two classes of neurons located distally and proximally in the neuronal cell layer. (D) Camera-lucida drawings of Golgi impregnated neurons innervating the peg sensilla (p). The somata in this figure are located at the base of the neuronal cell layer in the pectinal tooth. In these representative examples, 3-5 large neurons located in the inner nuclear layer innervated each peg sensillum. The image in panel C was constructed using Adobe PhotoShop (v.5). Four micrographs were taken at successive focal planes, digitized and then overlaid. Scale A-D = 10 μm.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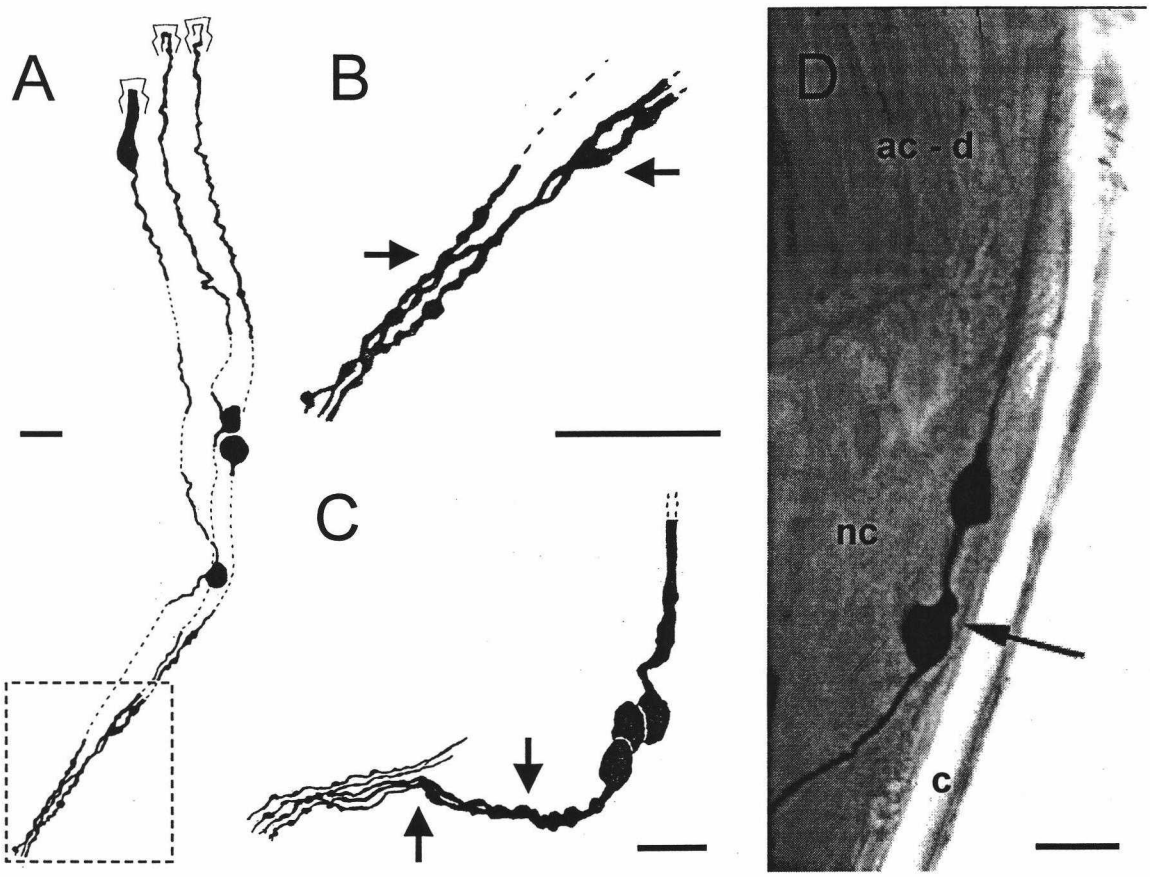
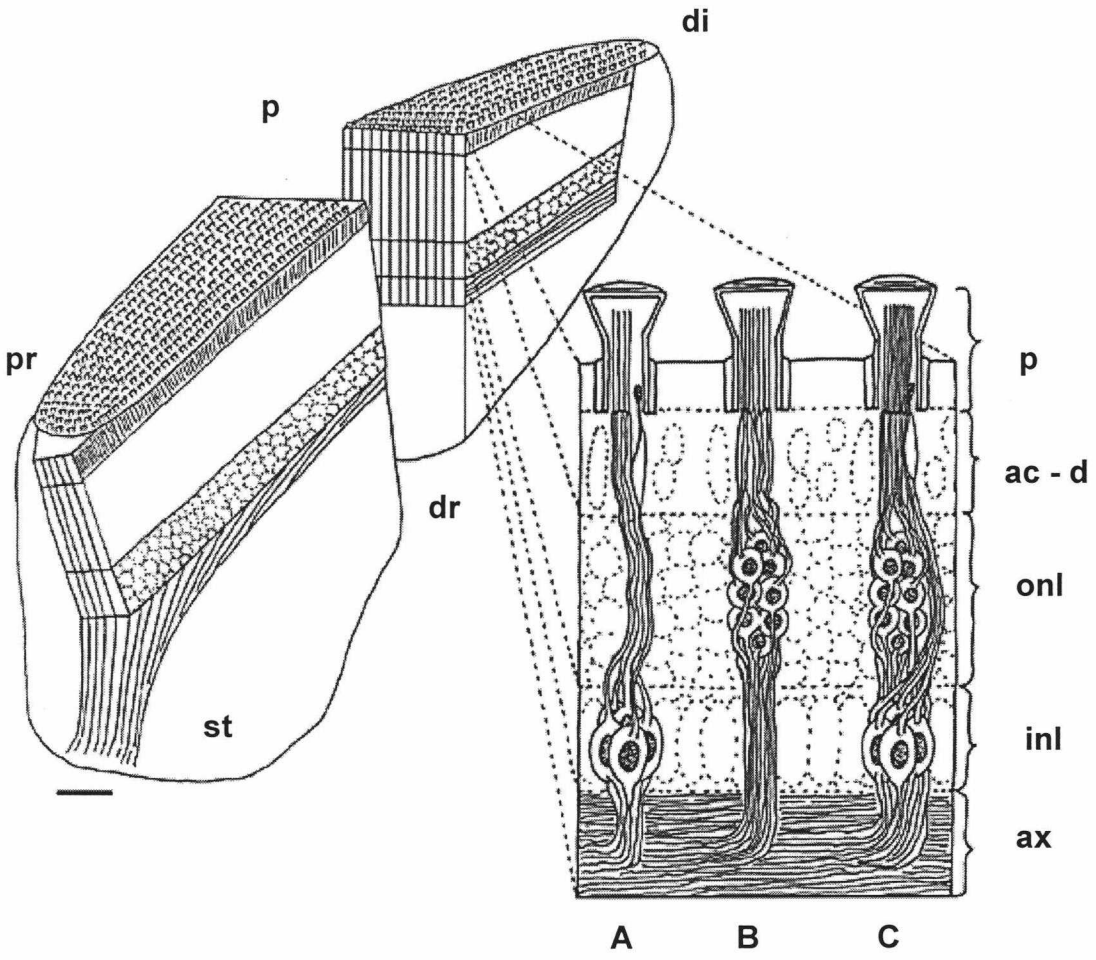


Figure 2.8 Different classes of axonal swellings within the pectinal teeth. (A) Camera-lucida drawing of three neighboring peg sensilla innervated by single silver impregnated sensory neurons. The sensory neurons observed in this preparation are located in the inner nuclear layer (INL) and were the only neurons impregnated within this pectinal tooth. (B) Magnified view of inset in panel A. Numerous axonal swellings form apparent axo-axonic contacts (arrows). (C) Camera-lucida drawing of three neurons in the INL innervating the same peg sensilla. Arrows show sites of axonal swellings. (D) Photograph of a Golgi-impregnated neuron forming an axonal swelling on the somata of another sensory neuron. The arrow indicates the point of apparent contact. Abbreviations: ac-d = accessory cell – dendritic layer, c = cuticle, nc = neuronal cell layer. Scale 10 μm.

Discussion

The ordered array of peg sensilla on the ventral surface of each pectinal tooth is associated with an ordered arrangement of sensory elements within the tooth (Fig 2.9). Three cytoarchitectural layers are evident beneath and parallel to the field of peg sensilla: a layer of sensory dendrites and accessory cells, a layer of neuronal somata and a plexiform layer of axonal processes. The neuronal cell layer is further divided into sub-layers of small and large neuronal somata defined as the outer and inner nuclear layers (ONL, INL). The dense fields of peg sensilla are innervated by cassettes of sensory neurons organized in vertical columns orthogonal to the histological layers described. Each sensillar cassette contains a stereotypical set of dendrites originating from the two classes of neurons in the ONL and INL that may respond to separate sensory modalities (Fig. 2.9 inset). The axonal layer below the two nuclear layers was immunoreactive to antibodies against the synaptic vesicle protein Synapsin, revealing small clusters of fluorescent labeling. Numerous axonal swellings resembling peripheral synapses were also observed in silver impregnated tissues between sensory axons in the axonal lamina and pectinal tooth nerve.

Figure 2.9 Summary diagram for proposed cytoarchitecture of sensory neurons innervating the peg sensilla on the pectinal teeth of scorpions.
Each pectinal tooth has three histological layers: an accessory cell-dendritic layer, a nuclear layer, and an axonal plexiform layer. The nuclear layer is divided into two sub-lamina (inner and outer nuclear layers) distinguished by soma size and innervation pattern. The axonal lamina below the nuclear layer resembles the plexiform layer of peripheral synapses described at the ultrastructural level by Foelix and Muller Vorholt (1983). It contains numerous axonal varicosities and is immunoreactive to antibodies against the synaptic vesicle protein Synapsin (not shown). Inset: The peg sensilla are innervated by parallel cassettes of sensory neurons. (A) The inner portion of the cassette is composed of a set of 3-5 bipolar neurons. One cell in this group has a dendrite that terminates at the base of the peg sensillum resembling a mechanoreceptor. (B) The outer portion of the cassette consists of many small bipolar neurons with dendrites that terminate in the shaft of the peg sensilla resembling chemoreceptors. (C) Diagram of a complete cassette of sensory neurons innervating one peg sensillum. Abbreviations: ac-d = accessory cell-dendritic layer, ax = axonal plexiform layer, dr = dorsal ridge, di = distal tip, i = inner nuclear layer, o = outer nuclear layer, p = peg sensilla, pr = proximal margin, st = stalk. Scale 10 μm for pectinal tooth. Inset of sensillar cassettes not drawn to scale.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The ultrastructure of the peg sensilla in *P. mesaensis* was similar to other species of scorpions (Ivanov and Balashov, 1979; Foelix and Muller-Vorholt, 1983) resembling other arthropod chemotactic setaform hairs (Slifer, 1970). The cassette of dendrites innervating each peg sensillum was composed of two types of dendrites (small and large) that were located in regular positions relative to an ensheathing microvillar cell. This polarity of dendrite size and placement is visible in other studies of peg sensilla ultrastructure (Ivanov and Balashov, 1979; Foelix and Muller-Vorholt, 1983).

Sensory neurons innervating the peg sensilla were located in two overlapping laminae that differed in cell size and innervation pattern suggesting that each sub-lamina may respond to chemical or mechanical stimuli. Dendrites from labeled cells in the ONL terminated within the shaft of the peg sensilla resembling other arachnid chemosensory neurons (Foelix and Chu-Wang, 1973b). Single dendrites originating from labeled cells in the INL terminated at the base of each peg sensillum, as are typical of arachnid mechanosensory neurons (Foelix and Chu-Wang, 1973a). While it appears that one cell in the INL may correspond to a mechanoreceptor, silver impregnated tissues suggest that another 3-5 cells in this region also innervate each peg sensillum. The function of these neurons is unknown but it is unlikely that more than one is mechanoreceptive (Foelix and Muller-Vorholt, 1983; Gaffin and Brownell, 1997a).

One possibility is that cells in the ONL converge on the 3-5 larger neurons in the INL. Two findings support this hypothesis: only 5 spike types have been

recorded in electrophysiological studies (Gaffin and Brownell, 1997a) and the number of axons in the pectinal tooth nerve is smaller than the estimated number of dendrites innervating the sensillar field (Brownell, per obs). This may explain qualitative differences in fluorescent labeling of the two laminae, as Neurobiotin is capable of transynaptic migration (Mesce et al., 1993).

The Axonal Plexiform Layer and Putative Synaptic Morphologies

The morphology and immunohistochemistry of the axonal tract below the neuronal layer suggests that this region contains the plexus of peripheral synapses discovered by Foelix and Muller-Vorholt (1983) in ultrastructural studies. The axonal lamina contained numerous punctate blebs that were immunoreactive to antibodies raised against the synaptic vesicle protein Synapsin (SYNORF1). This antibody labels neuropile in the scorpion cephalothoracic nerve mass (Melville, pers obs) and peripheral synapses in spider lyriform organs, tactile hairs, trichobothria, and internal joint receptors (Fabian-Fine et al., 1999a,b). Specific labeling of SYNORF1 has been observed at peripheral presynaptic terminals at the ultrastructural level (Fabian-Fine et al., 1999a). Western blots from preparations of scorpion CNS and the pectinal teeth indicate that a Synapsin-like protein is found in both tissues, consistent with our conclusion that the axonal lamina contains many peripheral synapses. In particular, immunoblots from the cephalothoracic nerve mass and pectinal teeth contained matching dominant bands at 70 kDa, near the molecular masses of *Drosophila* (70/74/80 kDa), rat (72/75 kDa) and spider (72/75

kDa) Synapsin isoforms (Volknandt et al., 1987; Klaggs et al., 1996; Fabian-Fine et al., 1999a). Several other matching bands were also visible (60 and 40 kDa) that may represent distinct isoforms of scorpion Synapsin.

The axonal appositions described in Golgi preparations may correspond to peripheral synapses known to occur in the pectinal teeth. At the ultrastructural level, both axo-somatic and axo-axonic synapses within the same sensillar cassette have been described (Foelix and Muller-Vorholt, 1983), suggesting that axonal swellings exhibiting this morphology in silver impregnated tissues may be peripheral synapses. Excitatory and inhibitory interactions between sensory cells innervating the peg sensilla have been observed using cross-correlation analysis. These intrasensillar interactions are not effected by transection of the pectinal nerve, suggesting that sensory afferents innervating the peg sensilla form local synaptic plexi involved in chemosensory processing (Gaffin and Brownell, 1997b).

Peripheral synapses are a common feature of Chelicerate sensory systems (Foelix, 1985b; Farley, 1999; Fahrenbach, 1999). Arachnid peripheral synapses were first described in the whip-like forelegs of whip-spiders (Arachnida: Amblpygi) (Foelix, 1975). The tip (tarsus) of each sensory whip supports thousands of chemosensory and tactile hairs. Axons from these tactile hairs form synaptic contacts on the dendrites, somata, and axons of a set of giant interneurons within the tarsus (Foelix and Troyer, 1980; Foelix, 1985b). These synaptic contacts are purely excitatory and mediate rapid withdrawal of the forelegs when the tarsus is touched (Igelmund and Wendler, 1991a,b). In spiders (Arachnida: Araneae),

peripheral synapses are found on the axons, somata and or dendrites of sensory neurons innervating tactile hairs, lyriform organs, and internal joint receptors (Foelix and Choms, 1979; Foelix, 1985b). A large proportion (> 50%) of these synaptic contacts are from inhibitory efferent fibers (Fabian-Fine et al., 1999a,b). It remains to be determined if afferent, efferent or both types of peripheral synapses are found in the pectinal teeth of scorpions (Arachnida: Scorpiones).

In merostomates (*Limulus*), synaptic plexi are found directly beneath the ommatidia of the compound eye (Fahrenbach, 1999 for review). A set of retinular cells and a single eccentric cell innervate each ommatidium. Axon collaterals from the eccentric cells form a meshwork of synaptic contacts involved in lateral inhibition (Ratliff et al., 1966; Hartline and Ratliff, 1974; Fahrenbach, 1985). Peripheral synaptic plexi are also associated with arrays of chemosensory sensilla on the gnathobases, flabellum, and book gill covers of horseshoe crabs (Hayes, 1971; Griffin and Fahrenbach, 1977; Hayes and Barber, 1982; see Fahrenbach, 1999 for review). The ancestral position of scorpions and merostomates in the sub-phylum Chelicerata (Stormer, 1969; Brusca and Brusca, 1990; Dunlop and Webster, 1999) suggests that peripheral synaptic plexi may be plesiomorphic character trait of early arachnids.

Functional Implications

Both the laminar and parallel arrangement of sensory afferents in the pectinal teeth of scorpions suggest that each tooth may be capable of detecting features of substrate texture and chemistry topographically. Each pectinal tooth supports a two-dimensional field of receptors, innervated by iterative cassettes of sensory neurons that respond to chemical and tactile stimuli (Gaffin and Brownell, 1997a). These sensory neurons are found in distinct laminae that form local networks of synapses (Foelix and Muller-Vorholt, 1983; Gaffin and Brownell, 1997b) and sensory afferents from the pectinal teeth are arranged topographically in sexually dimorphic neuropile in the cephalothoracic nerve mass (Brownell, 1989; Brownell, 1998). This chemosensory system shares organizational features with the compound eye of insects (Strausfeld and Blest, 1970; Strausfeld, 1970; Armett-Kibel et al., 1977) and the horseshoe crab *Limulus polyphemus* (Fahrenbach, 1975; Fahrenbach, 1985) suggesting that the pectines could function as a chemotactic retina.

Olfactory systems detect thousands of potential odorants in turbulent aerial or aquatic environments (Murlis and Jones, 1981; Atema, 1985) and appear to organize chemical information functionally rather than spatially (Homberg et al., 1989; Hannson et al., 1991, Hannson et al., 1992; Hildebrand and Shephard, 1997). Solid substrates may present opportunities for chemosensory signaling that are not present in viscous media like air and water as the physical shape of chemically

deposited signals can be fixed. The discrimination of qualitatively diverse chemical signals in conjunction with their spatial location is a complex problem (Laurent, 1999) that may require peripheral processing, underlying the role of excitatory and inhibitory interactions observed in the pectinal teeth (Gaffin and Brownell, 1997b).

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References

Abushama FT. 1964. The behavior and sensory physiology of the scorpion *Leiurus quinquestriatus*. *Anim Behav* 12:1140-153.

Alexander AJ. 1957. The courtship and mating behavior of the scorpion *Opisthophthalmus latimanus*. *Proc Zool Soc Lond* 128:529-44.

Alexander AJ. 1959. Courtship and mating in the buthid scorpions. *Proc Zool Soc Lond* 133:145-169.

Armett-Kibel C, Meinertzhagen IA, Dowling JE. 1977. Cellular and synaptic organization in the lamina of the dragon-fly *Sympetrum rubicundulum*. *Proc R Soc Lond B* 196:385-413.

Atema J. 1985. Chemoreception in the sea: adaptations of chemoreceptors and behavior to aquatic stimulus conditions. *Soc Exp Biol Symp* 39: 387-423.

Brownell PH. 1989. Neuronal organization and function of the pectinal sensory system in scorpions. *Soc Neurosi Abstr* 15:1289.

Brownell PH. 1998. Glomerular cytoarchitectures in chemosensory systems of arachnids. *Ann New York Acad Sci* 18:502-507

Brusca RC, Brusca GJ. 1990. *Invertebrates*. Sunderland: Sinauer Associates Inc.

Carthy JD. 1966. Fine structure and function of the sensory pegs on the scorpion pectine. *Experientia* 22:89-91.

Carthy JD. 1968. The pectines of scorpions. *Symp Zool Soc Lond* 23:251-261.

Cloudsey-Thompson JL. 1955. On the function of the pectines of scorpions. *Ann Mag Nat Hist* ser.12 8:556-60.

Daddow LYM. 1986. An abbreviated method of the double lead stain technique. *J Submircosc Cytol Pathol* 18: 221-224.

Dunlop JA, Webster M. 1999. Fossil evidence, terrestrialization and arachnid phylogeny. *J Arachnol* 27:86-93.

Fabian-Fine R, Hoger U, Seyfarth EA, Meinertzhagen IA. 1999a. Peripheral synapses at identified mechanosensory neurons in spiders: three-dimensional reconstruction and GABA immunocytochemistry. *J Neurosci* 19:298-310.

Fabian-Fine R, Volknandt W, Seyfarth EA. 1999b. Peripheral synapses at identifiable mechanosensory neurons in the spider *Cupiennius salei*: synapsin-like immunoreactivity. *Cell Tissue Res* 295:9-13.

Fahrenbach WH. 1975. The visual system of the horseshoe crab, *Limulus polyphemus*. *Int Rev Cytol* 41:285-349.

Fahrenbach WH. 1985. Anatomical circuitry of lateral inhibition in the eye of the horseshoe crab, *Limulus polyphemus*. *Proc R Soc Lond B* 225:219-249.

Fahrenbach WH. 1999. Merostomata. In: Harrison FW, Foelix RF, editors. *Microscopic anatomy of invertebrates. V8A: Chelicerate Arthropoda*. New York: Wiley-Liss. pp 1-115.

Farley RF. 1999. Scorpiones. In: Harrison FW, Foelix RF, editors. *Microscopic anatomy of invertebrates. V8A: Chelicerate Arthropoda*. New York: Wiley-Liss. pp 117-222.

Foelix RF, Chu-Wang I. 1973a. The morphology of spider sensilla: I. Mechanoreceptors. *Tissue Cell* 5:451-460.

Foelix RF, Chu-Wang I. 1973b. The morphology of spider sensilla: II. Chemoreceptors. *Tissue Cell* 5:461-478.

Foelix RF. 1975. Occurrence of synapses in peripheral nerves of arachnids. *Nature* 254:146-148.

Foelix RF, Choms A. 1979. Fine structure of a spider joint receptor and associated synapses. *Eur J Cell Biol* 19:149-159.

Foelix RF, Troyer D. 1980. Giant neurons and associated synapses in the peripheral nervous system of whip spiders. *J Neurocytol* 9:517-535.

Foelix RF, Muller-Vorholt G. 1983. The fine structure of scorpion sensory organs: II. Pectine sensilla. *Bull Brit Arachnol Soc* 6:68-74.

Foelix RF. 1985a. Mechano- and chemoreceptive sensilla. In: Barth FG, editor. *Neurobiology of Arachnids*. New York: Springer-Verlag. pp 118-137.

Foelix RF. 1985b. Sensory Nerves and Peripheral Synapses. In: Barth FG, editor. *Neurobiology of Arachnids*. New York: Springer-Verlag. pp 189-199.

Gaffin DD, Brownell PH. 1992. Evidence of chemical signaling in the sand scorpion, *Paruroctonus mesaensis* (Scorpionida: Vaejovidae). *Ethology* 91:59-69.

Gaffin DD, Brownell PH. 1997a. Response properties of chemosensory peg sensilla on the pectines of scorpiones. *J Comp Physiol (A)* 181:291-300.

Gaffin DD, Brownell PH. 1997b. Electrophysiological evidence of synaptic interactions within chemosensory sensilla of scorpion pectines. *J Comp Physiol (A)* 181:301-307.

Gaskell WH. 1902. The origin of vertebrates, deduced from the study of ammocoetes, Part X. *J Anat Lond* 36:164-208.

Griffin AJ, Fahrenbach WH. 1977. Gill receptor arrays in the horseshoe crab (*Limulus polyphemus*). *Tissue Cell* 9:745-750.

Hannson BS, Christensen TA, Hildebrand JG. 1991. Functionally distinct subdivisions of the macroglomerular complex in the antennal lobe of the male sphinx moth *Manduca sexta*. *J Comp Neurol* 312:264-278.

Hannson BS, Ljungberg H, Hallberg E, Lofstedt C. 1992. Functional specialization of olfactory glomeruli in a moth. *Science* 256:1313-1315.

Hartline HK, Ratliff F. 1974. Inhibitory interactions in the retina of Limulus. In: F Ratliff F, editor. *Studies on excitation and inhibition in the retina*. New York: Rockefeller Univ Press. pp 381-447.

Hayes WF. 1971. Fine structure of the Chemoreceptor sensillum in *Limulus*. *J Morphol* 133:205-240.

Hayes WF, Barber SB. 1982. Peripheral synapses in *Limulus* chemoreceptors. *J Comp Biochem Physiol* 72A:287-293.

Hildebrand JG. 1995. Analysis of chemical signals by nervous systems. *Proc Natl Acad Sci USA* 92:67-74.

Hildebrand JG, Shephard GM. 1997. Mechanisms of olfactory discrimination: converging evidence for common principles across phyla. *Annu Rev Neurosci* 20:595-631

Hjelle JT. 1990. Anatomy and morphology. In: Polis GA, editor. *The biology of scorpions*. Stanford: Stanford Univ Press. pp 9-63.

Hoffman C. 1964. Zur funktion der kammformigen organ von skorpionen. *Naturwissenschaften* 7:172.

Homberg U, Christensen TA, Hildebrand JG. 1989. Structure and function of the deuterocerebrum in insects. *Annu Rev Entomol* 34:477-501

Humphrey CD, Pittman FE. 1974. A simple methylene blue-azure II-basic fuschin stain for epoxy embedded sections. *Stain Technol* 42:9-14.

Igelmund P, Wendler G. 1991a. Morphology and physiology of peripheral giant interneurons in the forelegs (whips) of the whip spider *Heterophrynus elaphus* Pocock (Arachnida : Amblypygi). *J Comp Physiol (A)* 168:75-83.

Igelmund P, Wendler G. 1991b. The giant fiber system in the forelegs (whips) of the whip spider *Heterophrynus elaphus* Pocock (Arachnida : Amblypygi). *J Comp Physiol (A)* 168:63-73.

Ivanov VP, Balashov YS. 1979. The structural and functional organization of the pectine in the scorpion *Buthus eupews* studied by electron microscopy. In: Balalshov YS, editor. *The Fauna and Ecology of Arachnida.* Leningrad: Trudy Zool Inst. pp 73-87.

Klaggs BRE, Heimbeck G, Godenschwege TA, Hofbauer A, Pflugfelder GO, Reifegerste R, Reisch D, Schaupp M, Buchner S, Buchner E. 1996. Invertebrate synapsins: a single gene codes for several isoforms in *Drosophila. J Neurosci* 16:3154-3165.

Krapf D. 1986. Contact chemoreception of prey in hunting scorpions. (Arachnida : Scorpiones). *Zool Anz* 217:119-129.

Laurent G. 1999. A systems perspective on early olfactory coding. *Science* 286: 723-728.

Melville JM, Brownell PH. 1997. Laminar cytoarchitecture and topography of sensory neurons in the scorpion pectine. *Soc Neurosci Abstr* 23(1-2):768.

Melville JM, Tallarovic SK, Gundersen LE, and Brownell PH. 1999. Evidence of mate trailing in the giant hairy desert scorpion. *Anim Behav Soc Abstr* P68:53-54.

Mesce KA, Klukas KA, Brelje TC. 1993. Improvements for the anatomical characterization of insect neurons in whole mount: the use of cyanine-derived fluorophores and laser scanning confocal microscopy. *Cell Tissue Res* 271:381-397.

Murlis J, Jones CD. 1981. Fine scale structure of odor plumes in relation to insect orientation to distant pheromone and other attractant sources. *Physiol Entomol* 6:71-86.

Polis GA, Farley RD. 1979. Behavior and ecology of mating in the cannibalistic scorpion *Paruroctonus mesaensis* (Stahnke) (Scorpionida:Vaejovidae). *J Arachnol* 7:33-46.

Root TH. 1990. Neurobiology. In: Polis GA, editor. *The biology of scorpions*. Stanford: Stanford Univ Press. pp 341-409.

Schmidt M, Ache BW. 1996. Processing of antenullar input in the brain of the spiny lobster, *Panulirus argus*, II the olfactory pathway. *J Comp Physiol (A)* 178: 605-628.

Schneider D. 1992. 100 years of pheromone research. An essay on Lepidoptera. *Naturwissenschaften* 79:241-250.

Schroder O. 1908. Der sinnesorgane der skorpionskamme. *Z Wiss Zool* 9:436-444.

Sissom D. 1990. Systematics, biogeography and paleontology. In: Polis GA, editor. *The biology of scorpions*. Stanford: Stanford Univ Press. pp 161-223.

Slifer EH. 1970. The structure of arthropod chemoreceptors. *Annu Rev Entomol* 15:121-142.

Stahnke HL. 1972. UV light, a useful field tool. *Bioscience* 22:604-607.

Stormer L. 1969. Oldest known terrestrial arachnids. *Science*. 164:1276-1277.

Strausfeld NJ, Blest AD. 1970. Golgi studies on insects. Part I. The optic lobes of Lepidoptera. *Philosophic Trans R Soc Lond B* 258:82-134.

Strausfeld NJ. 1970. Golgi studies on insects. Part II. The optic lobes of Diptera. *Philosophic Trans R Soc Lond B* 258:135-223.

Strausfeld NJ. 1980. The Golgi method: Its application to the insect nervous system and the phenomenon of stochastic impregnation. In: Strausfeld NJ, Miller TA, editors. *Neuroanatomical techniques for the insect nervous system.* New York: Springer-Verlag pp. 132-190.

Swoveland MC. 1978. External morphology of the scorpion pectines. Masters thesis. California State University. San Francisco.

Volknandt W, Naito S, Ueda T, Zimmerman H. 1987. Synapsin I is associated with cholinergic nerve terminals in the electric organs of *Torpedo*, *Electrophorus*, and *Malapterus* and copurifies with *Torpedo* synaptic vesicles. *J Neurochem* 49:342-347.

Chapter 3

Peripheral Anatomy and Central Projections of Tactile Hairs on the Pectines of Scorpions

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Abstract

The comb-like pectines are unique sensory organs that scorpions (Arachnida: Scorpionida) use to monitor the mechanical and chemical properties of surfaces. These paired ventral appendages consist of an articulating spine and a row of cuticular teeth that intermittently brush the substrate. In desert sand scorpions (*Paruroctonus mesaensis*) sparse fields of sensory hairs (< 500) are located on the spine of the pectine and dense fields (> 5000) of small (< 7 μm) setaform sensilla called 'pegs' are found on the teeth (30-40 teeth / pectine). Here we describe the innervation pattern of sensory hairs on the pectinal spine, the internal architecture of the pectinal neuropile and compare the central projection pattern of tactile hairs on the pectinal spine to the bimodal (chemical and tactile) peg sensilla on the pectinal teeth. Long (> 400 μm) and medium (200-400 μm) length hairs on the pectinal spine resembled mechanoreceptors and short (< 200 μm) curved hairs resembled chemoreceptors. A synapse specific antibody (SYNORF1) clarified the internal architecture of the paired neuropile serving the pectines. Each pectinal neuropile was divided into a posterior laminar disk and an anterior cap. The cap of the pectinal neuropil was further divided into a fibrous cortex and medullar core containing numerous small glomeruli. Sensory afferents from tactile hairs on the pectinal spine were aligned somatotopically in the pectinal neuropile and were restricted to the outer cortex. Sensory afferents from the bimodal peg sensilla projected throughout the cap of the pectinal neuropile. This

pattern of innervation suggests that the pectinal neuropile may contain a neural map that encodes the fine textural features of substrates.

Introduction

The pectines of scorpions are paired comb-like organs attached ventrally to the second mesosomal segment (somite XI) and articulate from a point just posterior to the genital operculum (Hjelle, 1990). In desert sand scorpions (*Paruroctonus mesaensis*) each pectine has a jointed spine divided into marginal and medial lamellae that support 30-40 movable teeth (Swoveland, 1978; Melville and Brownell, 1997)(Fig 3.1a). The ventral surface of the pectine supports three populations of sensilla: (1) dense arrays of minute (< 5 μm) peg sensilla are restricted to the pectinal teeth, (2) sets of hairs located on dome-shaped structures called fulcra are found on the medial lamella (Fig 3.1b) and (3) a sparse population (< 200) of variable sensory hairs are distributed along the marginal lamella (Fig 3.1c)(Gaskell, 1902; Schroder, 1908; Carthy, 1966; Swoveland, 1978).

The peg sensilla have been the focus of several neuroanatomical and physiological studies (Hoffman, 1964, Ivanov and Balashov, 1979; Foelix and Muller-Vorholt, 1983; Gaffin and Brownell, 1997a,b). By ultrastructure and peripheral anatomy, the peg sensilla resemble chemosensory hairs found in arachnids and other arthropods (Slifer, 1970; Foelix and Chu-Wang, 1973a). Each sensillum is dually innervated by a single mechanosensory neuron and a set of chemosensory neurons (Ivanov and Balashov, 1979; Foelix and Muller-Vorholt, 1983; Gaffin and Brownell, 1997a). The biological functions of the peg sensilla are uncertain, although behavioral studies suggest they may be used to detect substrate

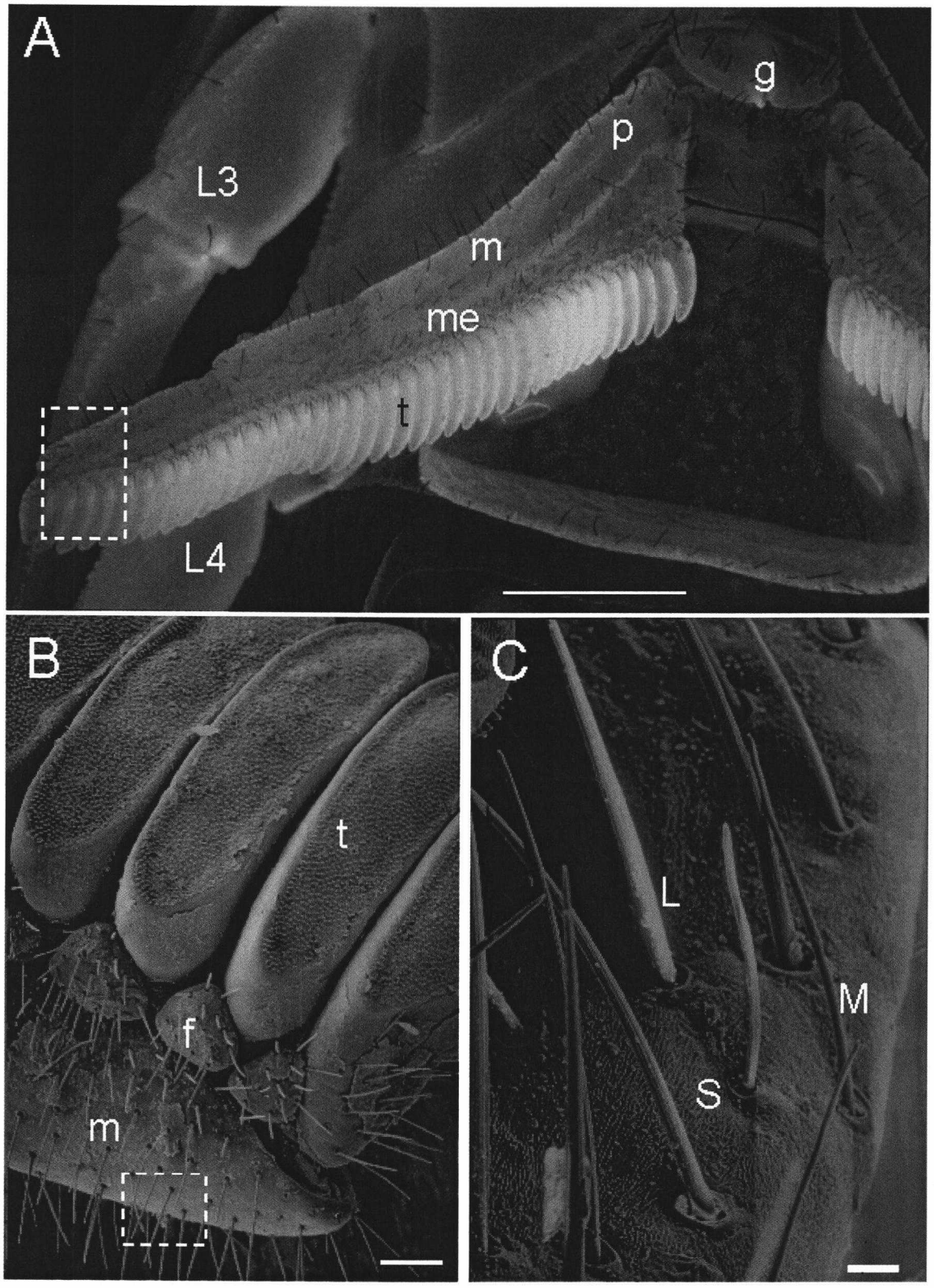
borne pheromones from prey and conspecifics (Krapf, 1986; Gaffin and Brownell, 1992; Melville et al., 1999). In contrast, the physiology and neuroanatomy of sensory hairs on the pectinal spine are unknown. Behavioral studies have demonstrated that the pectines are used to choose suitable substrates for both habitat and spermatophore deposition (Alexander 1957; 1959; Abushama, 1964). The sensory hairs on the pectinal spine or the peg sensilla on the pectinal teeth could serve this purpose (Abushama, 1964; Carthy, 1968).

Sensory afferents from the pectinal teeth project topographically to a pair of large ovoid neuropiles at the caudal end of the subesophageal nerve mass, forming a projection map of this receptor array (Brownell, 1998). This pattern of neural organization suggests that the pectines may encode the spatial distribution of chemical signals deposited on substrates. Previous studies have described the gross anatomy of the scorpion central nervous system (Hanstrom, 1923; Babu, 1965, Babu, 1985 for review) but the architecture of the pectinal neuropile has been investigated only recently (Brownell, 1989; 1998). The bimodal nature of the peg sensilla confounds the hypothesis that chemical information is represented somatotopically in the pectinal neuropile (Brownell, 1989; 1998). In both insects and arachnids, mechanosensory afferents often form somatotopic central projections (Ghysen, 1980; Murphey, 1981; Johnson and Murphy, 1985; Levine et al., 1985; Kent and Levine, 1988; Peterson and Weeks, 1988; Babu and Barth, 1989; Gronenburg, 1989; Anton and Barth, 1991; Newland, 1991; Gorb et al., 1993a). In flies and locusts, this includes mechanosensory afferents from gustatory

hairs and basiconic sensilla (Murphey et al., 1989; Newland, 2000). It remains to be determined if chemosensory, mechanosensory or both classes of afferents are aligned topographically in the pectinal neuropile of scorpions.

The objectives of the present study were to describe the following: the innervation pattern of sensory hairs on the pectinal spine, the architecture of the pectinal neuropile, and to compare the central projection pattern of sensory afferents from tactile hairs on the pectinal spine to the bimodal (chemical and tactile) receptors on the pectinal teeth. This was accomplished using standard immunological and neuroanatomical tracing methods coupled with confocal laser scanning microscopy.

Figure 3.1 External morphology of the pectinal spine.
(A) Ventral view of a desert sand scorpion (*P. mesaensis*) showing one of the comb-like pectines. The pectine (p) articulates from a joint just posterior to the gonopore (g) in a location close to the third (L3) and fourth (L4) pair of walking legs. The pectinal spine is composed of a medial (me) and marginal lamellae (m) that support the pectinal teeth (t). (B) Inset: Scanning electron micrograph of the terminal segment of the pectine. Sensory hairs are visible across the marginal lamella (m) and on turrets of cuticle called fulcra (f) on the medial lamella. The ventral surface of each pectinal tooth (t) supports hundreds of the short (> 5 μm) setaform sensilla. (C) Inset from B: Scanning electron micrograph of the marginal lamellae showing large (L) and medium (M) sensory hairs, and a short (S) curved sensory hair. Scale A = 2 mm, B = 100 μm, C = 10 μm.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Materials and Methods

Animals

Adult male and female sand scorpions (*P. mesaensis*) were collected in the Mojave Desert using portable blacklights (Stahnke, 1972) and maintained in the laboratory as described in the preceding chapter.

Neuronal Tracing Techniques

Anterograde labeling of sensory afferents was performed as described in the previous chapter. In brief, male sand scorpions (*P. mesaensis*) were immobilized, the pectinal nerve was transected and isolated and sensory afferents were labeled using the neuronal tracer Neurobiotin (Vector Labs). After labeling, the scorpions were anesthetized with CO_2 and the pectines were cut from the animal. The spines were then separated from the pectinal teeth and dissected into small sections (< 1 mm) with the anterior margin of the spine removed to maximize permeability. The tissues were fixed for 2-6 hours in 0.2M PBS containing 4% paraformaldehyde and 16% glutaraldehyde and then rinsed in 0.2M PBS (3 x 15 min.). To increase the permeability of the tissue the samples were placed in 0.2M PBS containing 3% Triton-X-100 for 1 hour, and then incubated in a 200/1 mixture of 0.2M PBS and strepavidin-Cy3 (Jackson Immuno Research Labs) for 24-48 hrs. Tissues were then rinsed in 0.2M PBS (3 x 15 min.) containing a 2000/1 dilution of DAPI

(Molecular Probes inc.) to label cell nuclei, dehydrated in serial alcohol dilutions, cleared in methyl salicylate and mounted in Cytoseal (Sigma inc.) for confocal observation.

Anterograde labeling methods for insect mechanosensory hairs (Gray and Weeks, 1999) were adapted for use in scorpions. Patches of long tactile hairs on the marginal lamellae were isolated in vaseline wells and then damaged with the aid of fine forceps (pulling) or metal hypodermic syringe (27 gauge) (scraping). The damaged area in the well was filled with a drop of distilled water for 10 seconds to aid in dendritic swelling and then withdrawn and replaced with scorpion saline and several small crystals of Neurobiotin. The wells were covered with vaseline to minimize evaporation and allowed to sit for 14-24 hours at room temperature. A similar technique was developed to label sensory afferents in the pectinal teeth. Sets of pectinal teeth (3 teeth per set) were isolated in a vaseline well. The ventral surface of each tooth was torn open using a metal syringe (27 gauge) or a sharpened tungsten needle (< 1 μm tip). Labeling of sensory afferents then proceeded as described above.

Following anterograde labeling, animals were anesthetized with CO_2 and the central nervous system was removed. The prosoma was cut free and placed into Millonigs buffer made isotonic with 0.2M sucrose. The cephalothoracic nerve mass was dissected free and the supra and subesophageal ganglia were separated by cutting through the circumesophageal connectives. These central nerve mass (CNS) preparations were fixed in 4% paraformaldehyde in 0.1M PBS for 2-6

hours, rinsed in 0.1M PBS (3 x 15 min), permeabilized in 8% Triton-X-100 in 0.2M PBS for 30 minutes and then placed in 3% Triton-X-100 in 0.2M PBS (2 x 30 min). CNS preparations were then incubated in strepavidin-Cy3, treated with DAPI, dehydrated, cleared, and whole mounted in Cytoseal (Sigma inc.).

Immunological methods

Immunohistochemical staining techniques were adapted from work on spider peripheral synapses (Fabian-Fine et al., 1999a,b). The cephalothoracic nerve mass was removed and then fixed in 0.1M PBS containing 4% paraformaldehyde for 1-2 hrs, washed in 0.1M PBS (3 x 15 min.), permeabilized in 3% Triton-X-100 in 0.1M PBS (3 x 30 min.) and pre-incubated in blocking medium (0.1M PBS with 5% nonfat milk powder and 0.1% TX-100) for 30 minutes. CNS preparations were then incubated for 48 hours at 4 degrees C in a 1:100 dilution of SYNORF1 (a monoclonal antibody raised against Drosophila-synapsin (Klaggs et al., 1996) containing blocking medium. CNS preparations were then washed in 0.1M PBS (3 x 15 min.) and incubated for 48 hours at 4 degrees C in blocking medium containing a 1:500 dilution of goat anti-mouse IgG coupled to Cy3 (Jackson Immuno Research Labs). CNS preparations were then treated with DAPI, dehydrated, cleared and mounted as described above.

Confocal Microscopy

Confocal images from whole mount preparations of pectinal spines and CNS preparations were captured using a Leitz DM IRBE inverted confocal scanning laser microscope (CSLM) as described in the previous chapter. All histological measurements were made using Scion Image software (version 3B).

Results

External Morphology and Distribution of Sensory Hairs on the Pectinal Spine

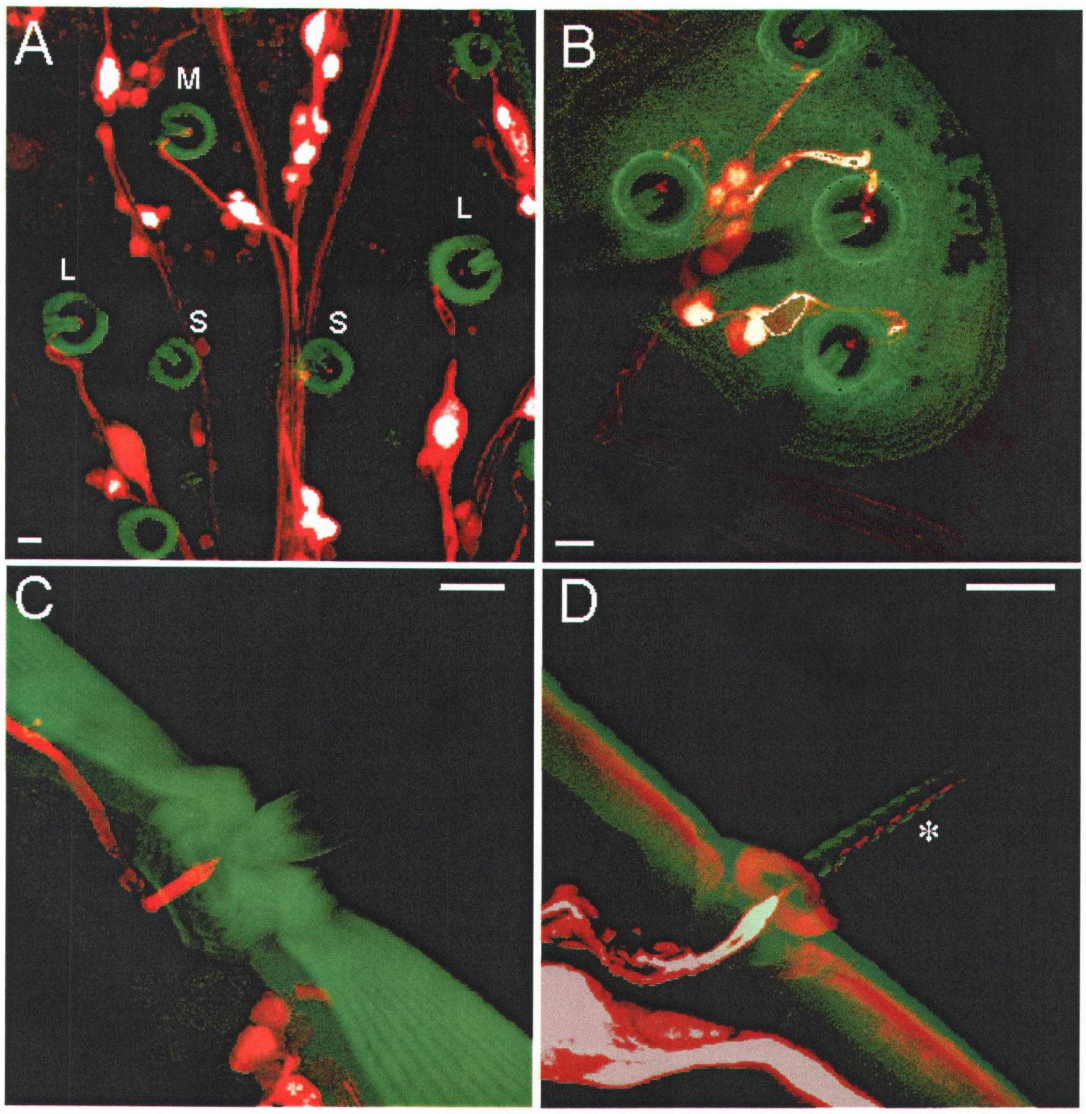
Sensory hairs on the pectinal spine were categorized by length into large (> 400 μm), medium (200-400 μm) and small (< 200 μm) sensilla as described by Swoveland (1978). The mean spacing interval for sensory hairs on the marginal lamellae was 90.5 μm (± 34.5 μm, n = 21). Shaft thickness was not indicative of hair length as the mean diameter of large and medium sensory hairs were both 10 μm (±3.2 μm, n = 11). Smaller sensory hairs were clearly distinguished by their curved shafts. The mean shaft diameter of the small hairs was 5.6 μm (±1.02 μm, n = 11). On the medial lamella, the mean spacing interval between sensory hairs was 33.4 μm (±10 μm, n = 28) and each fulcrum supported a set of 7 medium length sensory hairs on average (± 2.23 hairs, n = 53).

Peripheral Anatomy of Sensory Hairs

Large and medium sensory hairs on the marginal lamella were innervated by 2-6 bipolar neurons (avg. = 3.5 cells, ±1.05, n = 27) with mean cell diameters of 9 μm (±4.02 μm, n = 35). Small curved sensory hairs on the marginal lamellae were innervated by 5-14 bipolar neurons (avg. = 9.5 cells, ±2.18, n = 25) with mean cell diameters of 5 μm (±1.54, n = 50) (Fig 3.2a). On the medial lamella, medium sensory hairs on the fulcra were innervated by 2-6 bipolar neurons (avg. = 4, ±1.07,

n = 20) with mean cell diameters of 7 μm (±1.54 μm, n = 50) (Fig 3.2b). Dendrites innervating large and medium hairs terminated at the base of the sensillar socket (Fig 3.2c). Dendrites innervating small curved sensory hairs entered the hollow shaft (Fig 3.2d) in the manner typical of chemosensory hairs (Slifer, 1970). We classified large and medium sensory hairs on the pectinal spine as tactile sensilla and short curved hairs as chemosensory sensilla based on their innervation pattern.

Figure 3.2 Peripheral neuroanatomy of sensilla on the pectinal spine. (A) Sets of 1-3 bipolar neurons can be seen innervating two large (L) and one medium (M) sensory hair on the marginal lamella. A cluster of 3-4 bipolar neurons is also visible innervating a small (S) sensory hair. (B) Image of a fulcrum supporting four medium sensory hairs on the medial lamella. Each hair is innervated by 3-5 large (>10 μm) bipolar neurons. Sensory dendrites terminate at the base of each hair. (C) Dendritic innervation of a large sensory hair. Sensory dendrites (2-3) can be seen terminating at the base of the sensillar socket. (D) Dendritic innervation of a small curved sensory hair. Sensory dendrites are visible entering the base of the cuticular socket and continue through the hollow shaft (*) of the hair. Green = cuticle, red = sensory afferents. All panels are maximum projection images (M.P.I) with the following dimensions: M.P.I. for A = 16 sections through 61 μm, B= 16 sections through 12 μm, C = 10 sections through 33 μm, D = 12 sections through 34.5 μm. Scale A-D = 10 μm.


A
M
L
L
S
S
B
C
D
*

Topography of the Subesophageal Nerve Mass

The scorpion subesophageal nerve mass is a plurisegmental ganglion containing 6 pairs of neuromeres that are each associated with the pedipalps, walking legs or pectines (Babu, 1965; 1985; Hjelle, 1990). Each neuromere superficially resembles a ganglion, consisting of an outer rind of somata and a central neuropile (Babu, 1965; 1985; Root, 1990). The pectinal neuropils are ovoid in shape and are located at the caudal end of the subesophageal nerve mass. The anterior pectinal neuropiles are a set of smaller ovoid neuropils located anterior to the main pectinal neuropile (Brownell, 1998). Sets of transverse and longitudinal fiber tracts connect all the neuromeres of the subesophageal nerve mass. The longitudinal tracts are located at the midline and run along the anterior-posterior axis of the subesophageal nerve mass (Babu, 1965; 1985; Root, 1990). The longitudinal tracts are divided into dorsal and ventral sets of fibers. The dorsal tracts contain larger diameter fibers (6-10 μm) from motorneurons and interneurons. The ventral tracts consist of smaller axons from sensory afferents (Babu, 1965; 1985; Root, 1990). We have provided this description of the subesophageal ganglion to serve as reference for our anatomical findings.

Distribution of Synapsin-like Immunolabeling in the Subesophageal Nerve Mass and Pectinal Neuropile

CNS preparations treated with the monoclonal antibody SYNORF1 contained distinct fluorescent immunolabeling in all areas that have been identified as neuropile by conventional histological staining (Hanstrom, 1923; Babu, 1965; Brownell, 1998). Intense fluorescence was observed in the main and anterior pectinal neuropiles and associated neuromeres of the pedipalps and walking legs. Diffuse labeling was also observed in the paired longitudinal tracts along the ventral midline of the subesophageal nerve mass (Fig 3.3). The pectinal neuropiles extended 250-300 μm into the subesophageal nerve mass. Each neuropile was approximately 150 μm in diameter at its caudal base, 120 μm in diameter medially and 50 μm in diameter anteriorally. Proceeding anteriorally from the main pectinal neuropiles were parallel tracts of diffuse staining (175-200 μm long) that became continuous with the anterior pectinal neuropiles. The paired anterior neuropiles were ovoid in shape (40-50 μm x 60-75 μm) and abutted the posterior end of the ventral longitudinal tracts. Synapsin-like immunolabeling in the caudal region (first 50-60 μm) of the pectinal neuropile resembled a laminar disk while the anterior region (next 150-200 μm) resembled a cap. The cap was further divided into a fibrous outer cortex (20 μm thick) and an inner medullar region (90 μm in diameter) that contained small glomerular structures (Fig 3.4).

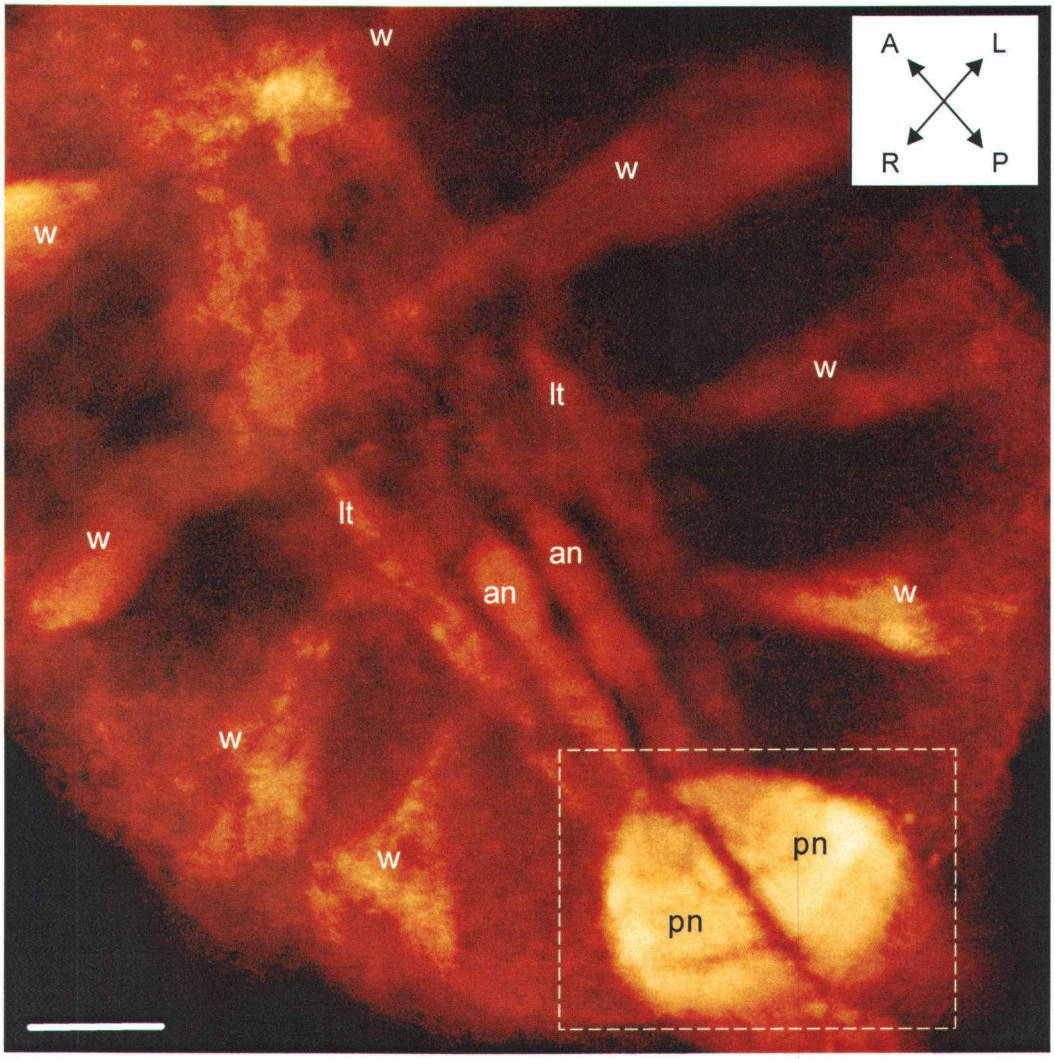


Figure 3.3 Neuromeres of the subesophageal nerve mass revealed by Synapsin-like immunofluorescence.
(A) Ventral view of the subesophageal ganglion. Specific immunofluorescence is visible in the pectinal neuropile (pn), anterior pectinal neuropile (an), lateral tracts (lt) and each neuromere of the walking legs (w). (B) Arrows indicate the (A) anterior - (P) posterior axis and (L) left and (R) sides of the subesophageal nerve mass. This image is an M.P.I. with the following dimensions: 40 sections through 100 μm. Scale = 100 μm

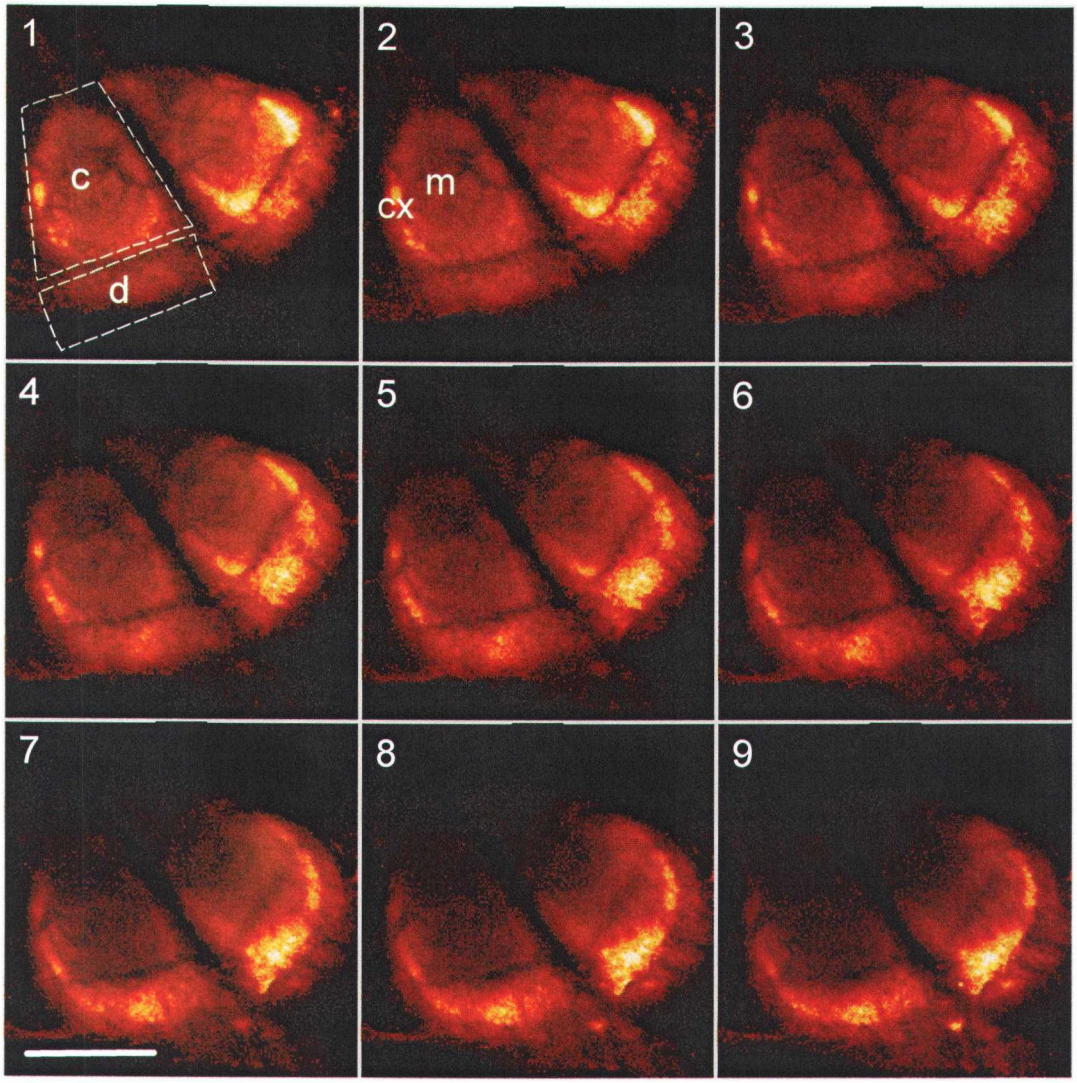


Figure 3.4 Internal architecture of the pectinal neuropile revealed by Synapsin-like immunofluorescence.
Inset from figure 3.3: Montage of 9 optical sections taken at 4 μm intervals through a SYNORF1 treated pectinal neuropile. The posterior region of the pectinal neuropile is laminar and disk-like (d). The anterior region resembles a cap (c). Beneath the outer cortex (cx) of the cap is a medullar region (me) composed of glomerular structures. Scale = 100 μm

Central Projections of Tactile Hair Afferents

Sensory afferents innervating tactile hairs on the pectinal spine were fluorescently labeled to determine how their axons were organized in the pectinal neuropile. Patches of damaged hairs (1-4) were distributed evenly from the base to terminal end of the pectine to label proximal, medial, and terminal sets of mechanosensory afferents. Sensory afferents from patches of damaged tactile hairs were associated with discrete fiber tracts in the pectinal nerve that projected ipsilaterally into the laminar disk and outer cortex of the cap before entering the anterior pectinal neuropile (Fig 3.5a). In all cases (n = 11), labeled afferents from tactile hairs were restricted to the outer cortex of the pectinal neuropil (Fig 3.5b). In a few cases (n = 4), labeled fibers from damaged tactile hairs projected through the longitudinal tracts from the anterior pectinal neuropile all the way forward to the base of the pedipalp neuromere (not shown). Sets of pectinal teeth were then damaged and labeled for comparative purposes. As previously shown by Brownell (1989; 1998), sensory afferents from the bimodal pectinal teeth projected into the both regions of the pectinal neuropil (Fig. 3.5c).

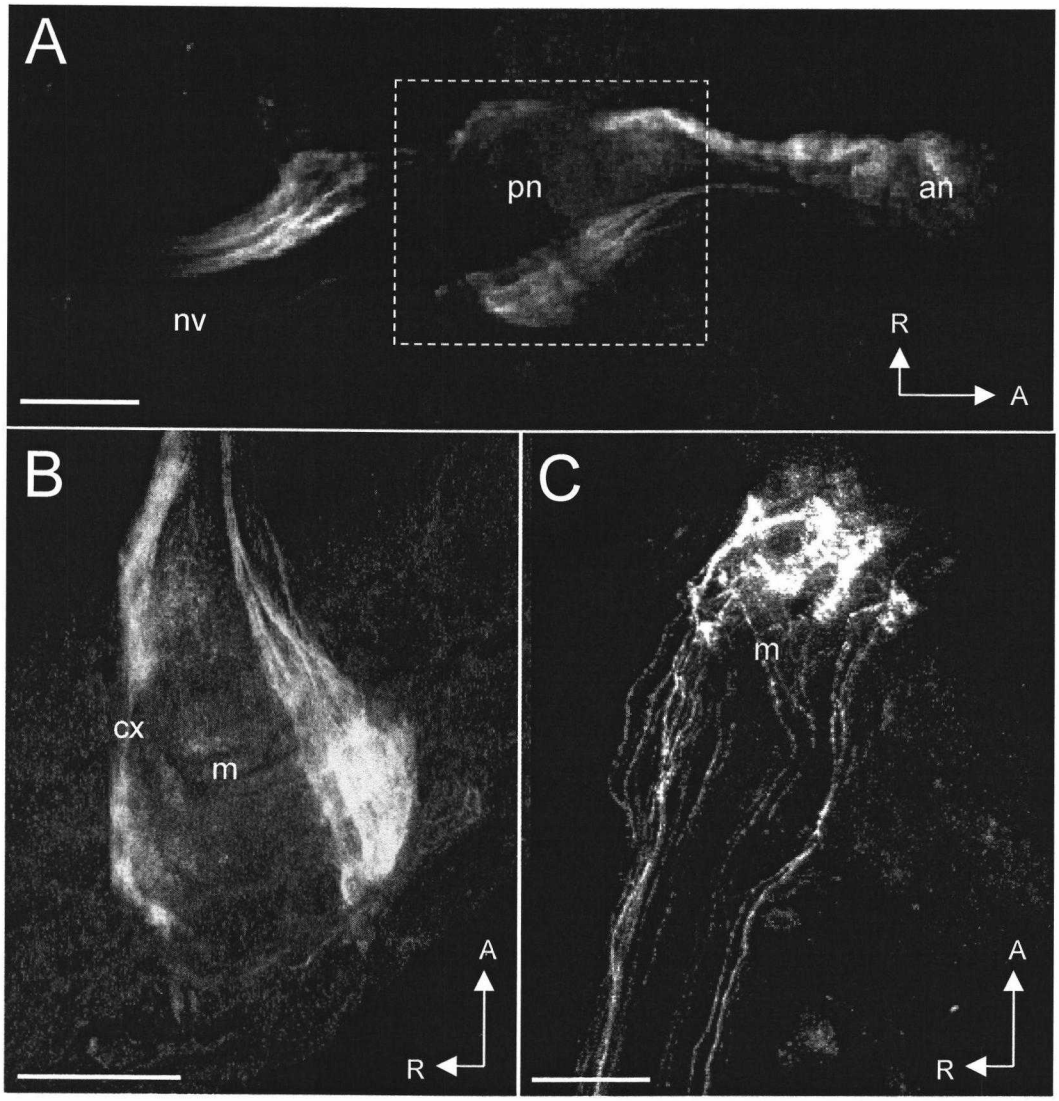
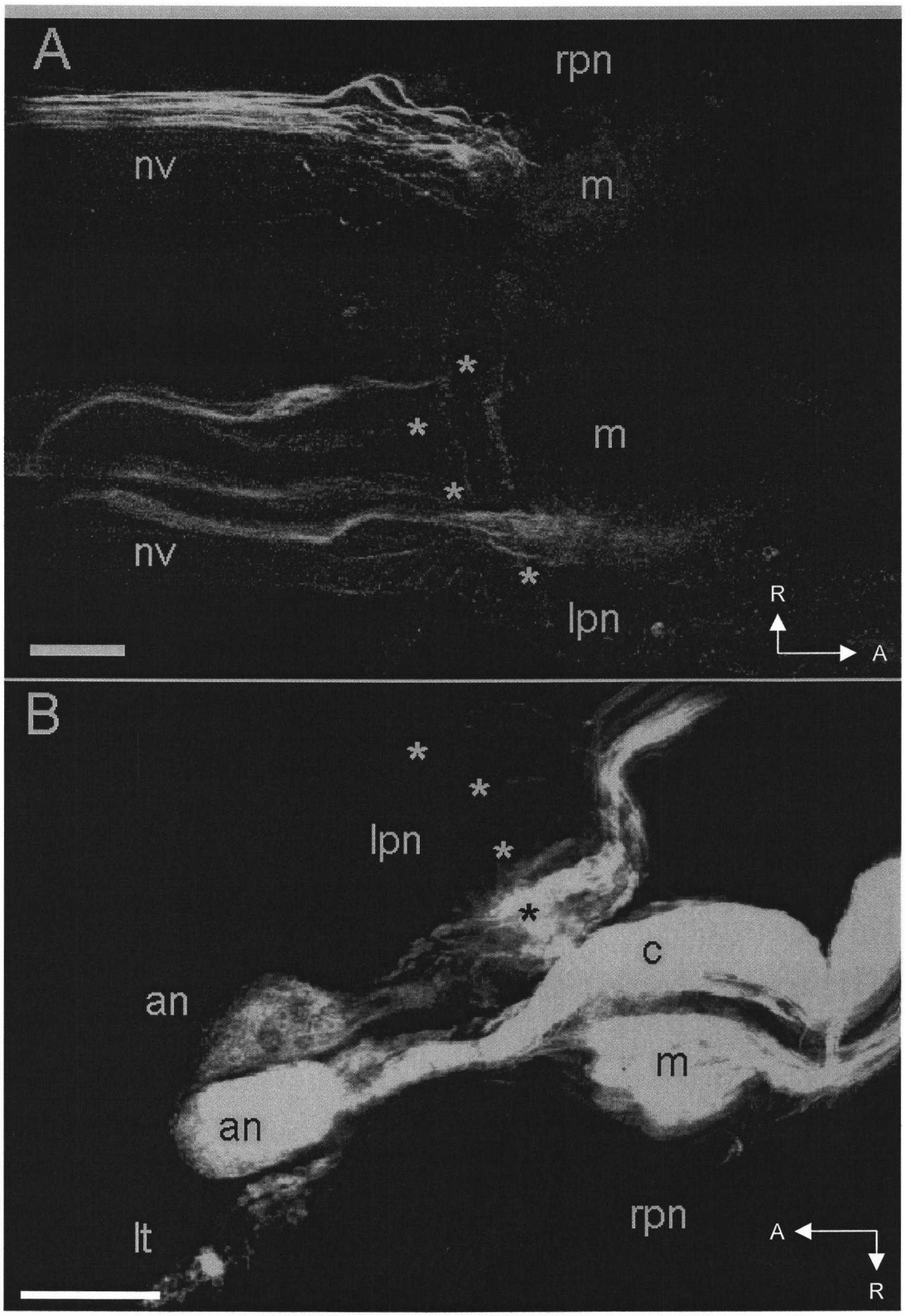


Figure 3.5 Central projection patterns of tactile hairs on the pectinal spine. (A) Labeled afferents from damaged tactile hairs are visible in the pectinal nerve (nv) as they project through the pectinal neuropile (pn) and into the anterior pectinal neuropile (an). (B) Inset from A at higher magnification. Labeled axons from tactile hairs are restricted to the outer cortex (cx) of the pectinal neuropil and are not visible in the medullar region (m). (C) Central projections patterns of bimodal receptors from the pectinal teeth. Fibers are visible in the pectinal nerve (nv) as they enter the medullar region (m) of the pectinal neuropil. Arrows point to (A) anterior and (R) right sides of the subesophageal nerve mass. Panels A-C = M.P.I. with the following dimensions: A = 45 sections through 161 um, B = 20 sections through 126 μm, C = 26 sections through 145 μm. Scale A-C 100 μm.

Sets of pectinal teeth and patches of tactile hairs on the pectine were damaged and labeled in a specific pattern to compare the central organization of their afferents. Sensory afferents from the pectines project ipsilaterally to the pectinal neuropil (Brownell, 1989; 1998). Sets of pectinal teeth were always damaged on the left pectine and tactile hairs were always damaged on the right pectine so their projections to the left and right pectinal neuropils could be compared simultaneously. Three sets of pectinal teeth were isolated and damaged to label distal, medial and proximal sets of bimodal sensory afferents (#10-12, 18-20, and 25-27 as sequentially numbered from the base to distal tip of the pectine). Patches of tactile hairs were damaged on the opposite pectine in equivalent positions to label matching sets of distal, medial and proximal mechanosensory afferents.

Labeled afferents from damaged pectinal teeth entered the laminar disk and projected into both regions of cap. Sensory afferents from tactile hairs on the pectinal spine projected in apparent topographic order through the laminar disk and cap but were restricted to the outer cortex (Fig 3.6A). In a few cases, brightly labeled afferents from the ablated pectinal teeth segregated into tracts that projected to both regions of the terminal cap. Afferents located superficially in the pectinal nerve were restricted to the cortex and afferents located medially projected into the medulla. Both tracts of afferents combined anteriorally to form a nerve that projected to the anterior pectinal neuropiles (Fig 3.6B).

Figure 3.6 Central projections of sensory afferents from mechanosensory hairs on the pectinal spine and mixed (chemosensory and mechanosensory) afferents from the peg sensilla on the pectinal teeth.
(A) Sets of labeled fibers from the pectinal teeth are visible in the pectinal nerve (nv) as they enter the right pectinal neuropile (rpn) and dive into the medullar (m) region of the cap. In contrast, sets of labeled fibers (*) from four patches of damaged mechanosensory hairs project through the left pectinal neuropile (lpn) with their position conserved. The fibers do not enter the medullar region (m). (B) Image of apparent mechanosensory and chemosensory pathways from the pectines. Bright labeling from ablated pectinal teeth can be seen in the cortex (c) and in the medullar region (m) of the right pectinal neuropile (rpn). Fibers then project to the anterior neuropile (an) and into the longitudinal tracts (lt). Arrows point to (A) anterior and (R) right sides of the subesophageal nerve mass. Panels A-B are M.P.I. with the following dimensions: A = 25 sections through 120 μm, B = 45 sections through 99 μm. Scale A-B = 100 μm.


A
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Discussion

The pectinal spine supports three types of sensory hairs with the following innervation patterns: long (> 400 μm) and medium (200-400 μm) length hairs were innervated at their base and short (< 200 μm) curved hairs contained dendrites in their shafts. The monoclonal antibody used in this study (SYNORF1) was raised against *Drosophila*-synapsin (Klaggs et al., 1996) and has been shown to label peripheral synapses in lyriform organs, tactile hairs, trichobothria, and internal joint receptors of a spiders (Fabian-Fine et al., 1999a,b). Synapsin-like immunolabeling was observed in the neuropiles of the cephalothoracic nerve mass of scorpions and clarified the internal architecture of the paired neuropile serving the pectines. Each pectinal neuropile consisted of a basal laminar disk and a terminal cap. The cap was further divided into a fibrous outer cortex and inner medullar region containing numerous small glomeruli. Mixed sensory afferents terminated in both regions of the terminal cap, but sensory afferents from tactile hairs were restricted to the cortex. Sensory afferents from tactile hairs on the pectinal spine were aligned in apparent somatotopic tracts that projected through the basal disk and cortex of the cap.

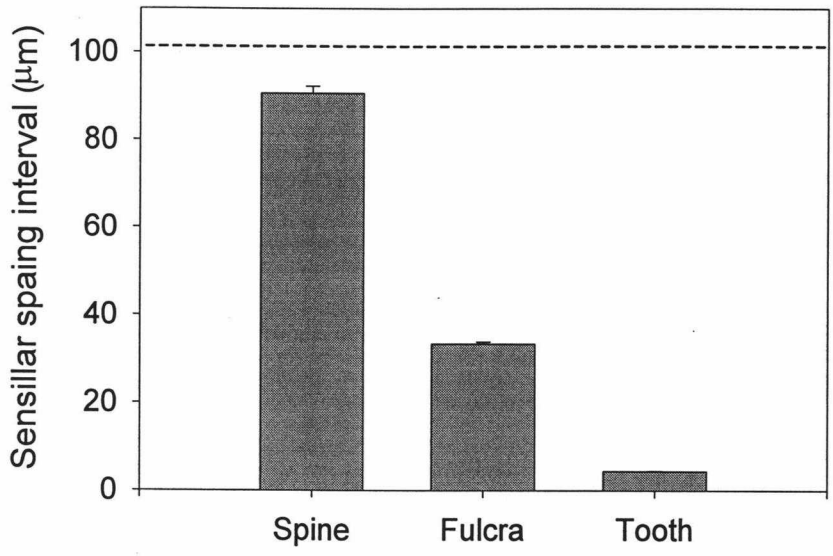
Large and medium hairs on the medial and marginal lamellae were innervated by a few cells with dendrites that terminated at the base of the hair, resembling tactile hairs of arachnids (Foelix and Chu Wang, 1973a). Small curved sensilla were innervated by numerous bipolar neurons with dendrites located in the

hollow shaft of the sensillum, resembling arachnid chemoreceptive hairs (Foelix and Chu Wang, 1973b). The pectinal spine supports approximately 200 sensory hairs (Swoveland, 1978) and at least 75% of the hairs are mechanoreceptive (Melville, pers obs). Based on these observations and the results presented (3 neurons per mechanosensory hair) the entire mechanosensory projection from the tactile hairs on both pectinal spines should contain fewer than 1000 mechanosensory afferents.

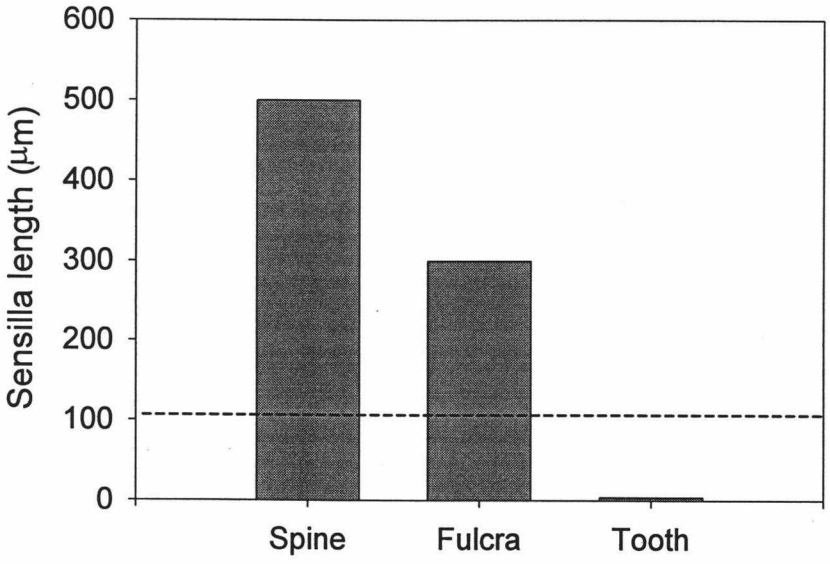
The tactile hairs on the pectinal spine were distributed in a manner consistent with sensing substrate texture (Fig 3.7). Dune substrates are composed of well-rounded sand grains with a median diameter of 150-200 μm (distribution = 50-500 μm). Desert sand grains are packed closely together making the substrate resemble a field of spheres. The average surface depth is then equal to the radius of a single grain (approx. 100 μm) (Brownell, 2000). The length (approx. 300 μm) and spacing interval (90 μm and 33 μm) of tactile hairs on the pectinal spine are within a range that could detect the textural features of a sandy dune environment. In contrast, the peg sensilla of the desert sand scorpion (*P. mesaensis*) are short pegs (< 4 μm) spaced at intervals 50 times closer than the diameter of a sand grain (Swoveland, 1978; Brownell, 1989, 2000). The high packing density and small length of the peg sensilla appears more consistent with a role in detecting fine chemical signals deposited on individual sand grains.

Figure 3.7 Comparison of sensillar spacing interval and sensilla length on each region of the pectine.
(A) Graph showing the mean distance between sensilla on the pectinal spine (spine), fulcra (fulcra), and the pectinal teeth (teeth). Bars = standard error. Dashed line = radius (100 μm) of an average sand grain (Brownell, 2000). (B) Graph showing the length of sensilla on the pectinal spine (spine), fulcra (fulcra), and the pectinal teeth (teeth). Values for sensilla lengths are from Swoveland (1978). Dashed line = radius (100 μm) of an average sand grain.

A. Spacing interval of sensilla on each region of the pectine



B. Length of sensilla on each region of the pectine



Behavioral experiments have shown that scorpions use the pectines to choose suitable substrates for burrowing and spermatophore deposition (Alexander, 1957; 1959; Abushama, 1964). In all of these studies, amputation, wax or varnish was used to eliminate all sensory input from the pectine, so tactile hairs on the pectinal spine, the peg sensilla or both types of sensilla may be involved in detecting surface textures (Abushama, 1964; Carthy, 1968). A series of behavioral experiments involving ablation of tactile hairs on the pectinal spine could readily resolve this question.

The outer cortex and medullar region of the terminal cap appear functionally divided into mechanosensory and chemosensory processing areas. The peg sensilla on the pectinal teeth contain chemosensory and mechanosensory neurons (Foelix and Mulller-Vorholt, 1983; Gaffin and Brownell, 1997a; Melville and Brownell, 1997). Sensory afferents from the pectinal teeth terminated in both the cortex and medullar region of the cap. In contrast, tactile afferents from the pectinal spine were restricted to the cortex. As previously observed by Brownell (1998), the medullar region of the terminal cap contained many small glomeruli that are a common structural feature of chemosensory processing areas in both arthropods and vertebrates (Shepard, 1994; Hildebrand, 1995; Hildebrand and Shephard, 1997 for review). The observed central projection pattern and internal structure of the pectinal neuropile is consistent with a functional separation of chemosensory and mechanosensory input to these two regions of the pectinal neuropile.

Mechanosensory afferents from the pectinal spine appeared to be aligned in somatotopic register as they entered the basal disk and outer cortex of the terminal cap. Distinct sets of fibers from damaged patches of mechanosensory hairs were found in the pectinal nerve. The number of labeled fiber tracts was consistent with the number of damaged patches of tactile hairs on each pectine, and the position of each tract of fibers remained constant as they projected through the pectinal neuropile. The preliminary results from this study suggest that mechanosensory information from the pectinal spine may be represented somatotopically in the pectinal neuropile. Brownell (1989, 1998) has observed that afferents from the chemosensory pectinal teeth also project topographically into the pectinal neuropile.

The paired antennae are the primary chemosensory appendages of mandibulate arthropods (crustaceans and insects). In insects, mechanosensory and olfactory afferents from the antennae project to two different areas of the insect brain: mechanosensory afferents project to the antennal mechanosensory and motor center (Strausfeld, 1976; Homberg et al., 1988; Homberg et al., 1989 for review) and chemosensory afferents project into the antennal lobe and terminate in numerous glomeruli (Hannson et al., 1991; 1992; Hildebrand, 1995 for review). Sensory afferents from the antennae are not somatotopically organized (Christensen et al., 1995; Hildebrand and Shephard, 1997) indicating that olfactory inputs are functionally, rather than spatially organized (Homberg et al., 1989; Hannson et. al., 1991; 1992).

Chemosensory systems that are spatially organized are rarely found in the animal kingdom. The extra-oral taste system of catfish (*Ictalurus* sp.) and the pectoral fin rays of the sea robin (*Prionotus carolinus*) are two vertebrate examples (Marui and Caprio, 1982; Finger, 1982; Hayama and Caprio, 1989; Finger, 1997, 2000). Sensory afferents from the extra-oral taste buds and tactile receptors from the lips, barbels and body surface of the catfish are organized somatotopically in the facial lobe (a specialized region of the medulla) forming overlapping chemical and tactile projection maps of the body surface (Marui and Caprio, 1982; Hayama and Caprio, 1989). The fin rays of the sea robin are ventro-lateral extensions of the pectoral fin that support dense arrays of solitary chemoreceptor cells (Morrill, 1895, Whitear 1971). These ventrally projecting chemosensory appendages are used to find prey (Scharrer et al., 1947; Barbach and Case, 1965; Silver and Finger, 1984). Spinal nerves innervating each ray contain chemosensory, tactile, and proprioreceptive afferents that project to corresponding lobules in the dorsal horn of the spinal cord (Morril1, 1895; Herrick, 1907). The lobules are aligned somatotopically in a reversed anterior to posterior order (Finger, 1982; Finger, 1997 for review). Ascending fibers from the lobule of each fin ray project to the cerebellum and optic tectum, suggesting that chemical and tactile information from the fin rays may be represented somatotopically in higher brain regions (Finger, 2000).

Similar contact chemosensory systems have recently been described in arachnids and insects (Brownell 1998, Newland et al. 2000). The peg sensilla of

scorpions and the basiconic sensilla of locusts are similar in structure and function: both sensilla are innervated by a single mechanosensory neuron and a set of chemosensory neurons (Blaney and Chapman, 1969; Klein, 1980; Foelix and Muller-Vorholt, 1983; Melville and Brownell, 1997). In locusts, sensory afferents from basiconic and tactile sensilla on the legs form two parallel projection maps in the mesothoracic ganglion (Newland, 2000). Sensory afferents from locust gustatory sensilla do not appear to terminate in modality specific regions of the thoracic neuropil (Newland, 2000). This is contrary to the well-established tarsal taste system of flies (Murphey et al., 1989).

The pectinal neuropil shares certain organizational features with the tarsal taste systems of both locusts and flies: sensory afferents from the pectine project to modality specific regions of the pectinal neuropile and our preliminary analysis suggests that both of these projections are aligned in somatotopic register (Brownell, 1998; and data presented here). This pattern of innervation suggests that the pectinal neuropile may contain two neural maps that encode fine textural (cortex) and chemical (medulla) features of substrates.

Acknowledgments

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References

Abushama FT. 1964. The behavior and sensory physiology of the scorpion *Leiurus quinquestriatus* (H. & E). *Anim Behav* 12:1140-153.

Alexander AJ. 1957. The courtship and mating of the scorpion *Opisthophthalmus latimanus*. *Proc Zool Soc Lond* 128:529-44.

Alexander AJ. 1959. Courtship and mating in the buthid scorpions. *Proc Zool Soc Lond* 133:145-169.

Anton S, Barth FG. 1993. Central nervous projection patterns of trichobothria and other cuticular sensilla in the wandering spider *Cupiennius salei* (Arachnida, Araneae). *Zoomorphology* 217:129-136.

Babu KS. 1965. Anatomy of the central nervous system of arachnids. *Zool Jahrb Anat* 82:1-154.

Babu KS. 1985. Patterns of arrangement and connectivity in the central nervous system of arachnids. In: Barth FG, editor. *Neurobiology of Arachnids*. New York: Springer-Verlag. pp 3-19.

Babu KS, Barth FG. 1989. Central nervous projections of mechanoreceptors in the spider *Cupiennius salei*. *Cell Tissue Res* 258:69-82.

Barbach JE, Case J. 1965. Sensory capabilities of the modified fins of the squirrel hake (*Urophycus chus*) and sea robins (*Prionotus carolinus* and *P. evolans*). *Copeia* 2:194-206.

Blaney WM, Chapman RF. 1969. The anatomy and histology of the maxillary palp of the locust, *Schistocerca gregaria* (Orthoptera, Acrididae). *J Zool* 157:509-535.

Brownell PH. 1989. Neuronal organization and function of the pectinal sensory system in scorpions. *Soc Neurosci Abstr* 15:1289.

Brownell PH. 1998. Glomerular cytoarchitectures in chemosensory systems of arachnids. *Ann New York Acad Sci* 502-507.

Brownell PH. 2000. Sensory ecology and orientational behaviors. In: Brownell PH, Polis G, editors. *Scorpion Biology and Research*. New York: Oxford Univ Press. pp 159-183.

Carthy JD. 1966. Fine structure and function of the sensory pegs on the scorpion pectine. *Experientia* 22:89-91.

Carthy JD. 1968. The pectines of scorpions. *Symp Zool Soc Lond* 23:251-261.

Christensen TA, Hildebrand JG. 1987. Functions, organization, and physiology of the olfactory pathways in the lepidoteran brain. In: Gupta AP, editor. *The Arthropod Brain: its evolution, development, structure and functions*. New York: John Wiley and Sons. pp 457-483.

Christensen TA, Harrow ID, Cuzzocrea C, Randolph PW, Hildebrand JG. 1995. Distinct projections of two populations of olfactory receptor axons in the antennal lobe of the sphinx moth *Manduca sexta*. *Chem Senses* 20: 313-323.

Fabian-Fine R, Hoger U, Seyfarth EA, Meinertzhagen IA. 1999a. Peripheral synapses at identified mechanosensory neurons in spiders: three-dimensional reconstruction and GABA immunocytochemistry. *J Neurosci* 19:298-310.

Fabian-Fine R, Volknandt W, Seyfarth EA. 1999b. Peripheral synapses at identifiable mechanosensory neurons in the spider *Cupiennius salei*: synapsin-like immunoreactivity. *Cell Tissue Res* 295:9-13.

Finger TE. 1982. Somatotopy in the representation of the pectoral fin and free fin rays in the spinal cord of the sea robin, *Prionotus carolinus*. *Biol Bull* 163:154-161.

Finger TE. 1997. Evolution of taste and solitary chemoreceptor cell systems. *Brain Behav Evol* 50:234-243.

Finger TE. 2000. Ascending spinal systems in the fish, *Prionotus carolinus*. *J Comp Neurol* 422:106-122.

Foelix RF, Chu-Wang I. 1973a. The morphology of spider sensilla. I. Mechanoreceptors. *Tissue Cell* 5:451-460.

Foelix RF, Chu-Wang I. 1973b. The morphology of spider sensilla. II. Chemoreceptors. *Tissue Cell* 5:461-478.

Foelix RF, Muller-Vorholt G. 1983. The fine structure of scorpion sensory organs. II. Pectine sensilla. *Bull Brit Arachnol Soc* 6:68-74.

Foelix RF. 1985. Mechano and chemoreceptive sensilla. In: Barth FG, editor. *Neurobiology of Arachnids*. New York: Springer-Verlag. pp 118-137.

Gaffin DD, Brownell PH. 1992. Evidence of chemical signaling in the sand scorpion, *Paruroctonus mesaensis* (Scorpionida: Vaejovidae). *Ethology* 91:59-69.

Gaffin DD, Brownell PH. 1997a. Response properties of chemosensory peg sensilla on the pectines of scorpiones. *J Comp Physiol (A)* 181:291-300.

Gaffin DD, Brownell PH. 1997b. Electrophysiological evidence of synaptic interactions within chemosensory sensilla of scorpion pectines. *J Comp Physiol (A)* 181:301-307.

Gaskell WH. 1902. The origin of vertebrates, deduced from the study of ammocoetes. Part X, *J Anat Lond* v36:164-208.

Ghysen A. 1980. The projection of sensory neurons in the central nervous system of *Drosophila*: choice of the appropriate pathway. *Dev Biol* 78:521-541.

Gorb SN, Anton S, Barth FG. 1993. Central projections of cheliceral mechanoreceptors in the spider *Cupiennius salei* (Arachnida, Araneae). *J Morpholology* 217:129-136.

Gray JR, Weeks JC. 1999. Anatomical correlates of a steroid induced change in synaptic strength during development. *Soc Neurosci Abstr* 25(1-2):124.

Gronenburg W. 1989. Anatomical and physiological observations on the organization of mechanoreceptors and local interneurons in the central nervous system of the wandering spider *Cupiennius salei. Cell Tissue Res* 258:163-175.

Hannson BS, Christensen TA, Hildebrand JG. 1991. Functionally distinct subdivisions of the macroglomerular complex in the antennal lobe of the male sphinx moth *Manduca sexta. J Comp Neurol* 312:264-278.

Hannson BS, Ljungberg H, Hallberg E, Lofstedt C. 1992. Functional specialization of olfactory glomeruli in a moth. *Science* 256:1313-1315.

Hanstrom B. 1923. Further notes on the central nervous system of arachnids: scorpions, phalangids and trap door spiders. *J Comp Neurol* 35:249-272.

Hayama T, Caprio J. 1989. Lobule structure and somatotopic organization of the medullary facial lobe in the channel catfish *Ictalurus punctatus. J Comp Neurol* 285:9-17.

Herrick CJ. 1907. The tactile centers in the spinal cord and brain of the sea robin, *Prionotus carolinus. J Comp Neurol Psychol* 17:307-327.

Hildebrand JG. 1995. Analysis of chemical signals by nervous systems. *Proc Natl Acad Sci USA* 92: 67-74.

Hildebrand JG, Shephard GM. 1997. Mechanisms of olfactory discrimination: converging evidence for common principles across phyla. *Annu Rev Neurosci* 20:595-631.

Hjelle JT. 1990. Anatomy and morphology. In: Polis GA, editor. *The biology of scorpions*. Stanford: Stanford Univ Press. pp 9-63.

Hoffman C. 1964. Zur funktion der kammformigen organ von skorpionen. *Naturwissenschaften* 7:172.

Homberg U, Montague RA, Hildebrand JG. 1988. Anatomy of the antenno-cerebral pathways in the brain of the sphinx moth *Manduca sexta*. *Cell Tissue Res* 254:255-281.

Homberg U, Christensen TA, Hildebrand JG. 1989. Structure and function of the deuterocerebrum in insects. *Annu Rev Entomol* 34:477-501.

Ivanov VP, Balashov YS. 1979. The structural and functional organization of the pectine in a scorpion *Buthus eupews* studied by electron microscopy. In: Balalshov YS, editor. *The Fauna and Ecology of Arachnida*, Leningrad: Trudy Zool Inst. pp 73-87.

Johnson SE, Murphy RK. 1985. The afferent projection of mesothoracic bristle hairs in the cricket, Acheta domesticus. *J Comp Physiol (A)* 156:369-379.

Kent KS, Levine RB. 1988. Neural control of leg movements in a metamorphic insect: sensory and motor elements of the larval thoracic legs in *Manduca sexta*. *J Comp Neurol* 271:559-576.

Klaggs BRE, Heimbeck G, Godenschwege TA, Hofbauer A, Pflugfelder GO, Reifegerste R, Reisch D, Schaupp M, Buchner S, Buchner E. 1996. Invertebrate synapsins: a single gene codes for several isoforms in *Drosophila*. *J Neurosci* 16:3154-3165.

Klein U. 1980. Sensilla of the cricket palp. Fine structure and spatial organization. *Cell Tissue Res* 219:229-252.

Krapf D. 1986. Contact chemoreception of prey in hunting scorpions (Arachnida: Scorpiones). *Zool Anz* 217:119-129.

Levine RB, Pak C, Linn D. 1985. The structure, function and metamorphic reorganization of somatotopically projecting sensory neurons in *Manduca sexta* larvae. *J Comp Physiol (A)* 157:1-13.

Marui T, Caprio J. 1982. Electrophysiological evidence for the topographical arrangement of taste and tactile neurons in the facial lobe of the channel catfish. *Brain Res* 231:185-190.

Melville, JM, Brownell PH. 1997. Laminar cytoarchitecture and topography of sensory neurons in the scorpion pectine. *Soc Neurosci Abstr* 23(1-2):768.

Melville JM, Tallarovic SK, Gundersen LE, and Brownell PH. 1999. *Anim Behav Soc Abstr* P38.53-54.

Morrill AD. 1895. The pectoral appendages of *Prionotus* and their innervation. *J Morphol* 11:177-192.

Murlis J, Jones CD. 1981. Fine scale structure of odor plumes in relation to insect orientation to distant pheromone and other attractant sources. *Physiol Entomol* 6:71-86.

Murphey RK. 1981. The structure and development of a somatotopic map in crickets: the cercal afferent projection. *Dev Biol* 88:236-246.

Murphey RK, Possidente D, Pollack G, Merritt DJ. 1989. Modality specific axonal projections in the CNS of the flies *Phormia* and *Drosophila*. *J Comp Neurol* 290:185-200.

Newland PL. 1991. Morphology and somatotopic organization of the central projections of afferents from tactile hairs on the hind leg of the locust. *J Comp Neurol* 311:1-16.

Newland PL, Rogers SM, Gaaboub I, Matheson T. 2000. Parallel somatotopic maps of gustatory and mechanosensory neurons in the central nervous system of an insect. *J Comp Neurol* 425:82-96.

Peterson BA, Weeks JC. 1988. Somatotopic mapping of sensory neurons innervating mechanosensory hairs on the larval prolegs of *Manduca sexta*. *J Comp Neurol* 275:128-144.

Root TH. 1990. Neurobiology. In: Polis GA, editor. *The biology of scorpions*. Stanford: Stanford Univ Press. pp 341-409.

Scharrer E, Smith SW, Palay SL. 1947. Chemical senses and taste in the fishes *Prionotus* and *Trichogaster*. *J Comp Neurol* 86:183-198.

Schroder O. 1908. Der sinnesorgane der skorpionskamme. *Z Wiss Zool* 9:436-444.

Shepherd GM. 1994. Discrimination of olfactory signals by the olfactory receptor neuron. *Neuron* 13:771-790.

Silver WL, Finger TE. 1984. Electrophysiological examination of a non-olfactory, non-gustatory chemosense in the sea robin, *Prionotus carolinus*. *J Comp Physiol (A)* 154:167-174.

Slifer EH. 1970. The structure of arthropod chemoreceptors. *Annu Rev Entomol* 15:121-142.

Stahnke HL. 1972. UV light, a useful field tool. *Bioscience* 22:604-607.

Strausfeld NJ. 1976. *Atlas of an insect brain*. Heidleberg: Springer-Verlag.

Swoveland MC. 1978. External morphology of the scorpion pectines. Masters thesis, California State University, San Francisco.

Whitear M. 1971. Cell specialization and sensory function in fish epidermis. *J Zool Lond* 163:237-264.

Chapter 4

Evidence of Mate Trailing in the Giant Hairy Desert Scorpion

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Abstract

During the mating season, male desert scorpions are often found wandering through dune environments, presumably in search of female conspecifics. For a male desert scorpion, finding a receptive female is a potentially difficult and hazardous task, suggesting that wandering by the male may be a form of guided mate-searching. In this study we examined whether male giant hairy desert scorpions (*Hadrurus arizonensis*) were capable of trailing female conspecifics using a Y-maze choice test. In three separate experiments, a significant proportion of male scorpions preferred the arm of the maze that a reproductive female scorpion had walked down. Male scorpions did not prefer maze arms that male conspecifics had walked down, indicating that this response was sex-specific. When the substrate was repeatedly exposed to a reproductive female, males took significantly longer to complete the Y-maze test, exhibited an increase in pausing behavior, and displayed juddering and tail-wagging responses that have been described as pre-courtship behaviors in this and other species of scorpions. A novel lunging behavior was also expressed, resembling sex-specific mate seizing behavior. Substrates repeatedly exposed to other males did evoke juddering, but at very low frequencies (< 5%). In contrast, tail wagging and lunging were only elicited when the substrate was exposed to a reproductive female. The results from this study suggest that male *H. arizonensis* can orient and respond to substrate borne signals from female conspecifics that are likely chemical in origin.

Introduction

Finding a mate can be a difficult task for solitary and nocturnal animals and often requires the emission, transmission, and reception of signals that are sex specific (Dusenberry, 1993; Dryer and Brockman, 1996 for reviews). In many mating systems sexual communication is mediated by chemical signals (sex pheromones) (Carde and Baker, 1984; Abelson, 1985; Mason, 1993) although acoustic (Walker, 1957; Ewing, 1989 for review) visual and vibratory signals can serve a similar function (Lewis and Gower, 1980; Lloyd, 1983; Rovner and Barth, 1981; Barth, 1985). Arthropods are exceptional models for investigating the role that sex pheromones play in sexual communication with regard to mate finding and courtship (Carde and Baker, 1984; Bell et al., 1995). With the discovery of over 1,000 insect sex pheromones (Abelson, 1985) it is remarkable that so little is known about chemical communication in the second largest group of terrestrial arthropods, the arachnids. To date, only two sex pheromones have been isolated in this class of animals (Berger, 1972; Schultz and Toft, 1993). This may be due in part to the prevailing hypothesis at the turn of the century that chemical signaling was minimized in arachnids (Warburton, 1909). The dominant cues in arachnid mate finding and courtship were assumed to be visual or tactile, as evidenced by the apparent lack of specialized chemosensory appendages (antennae) in this class of animals. It was not until the latter half of this century that studies on ticks (see Sonenshine, 1985 for review) and spiders (see Tietjen and Rovner, 1982; Pollard et

al., 1987 for reviews) demonstrated the wide use of pheromonal signaling in arachnids.

A growing body of evidence suggests that scorpions also use chemical cues to initiate courtship and find potential mates (see Gaffin and Brownell, 2000 for review). When placed on substrates previously occupied by female conspecifics, male scorpions exhibit changes in walking or locomotor pattern and will express juddering and tail-wagging responses that may be pre-courtship behaviors (Krapf, 1986; Gaffin and Brownell, 1992). This suite of behaviors is also evoked when male sand scorpions encounter sand treated with hexane or methylene chloride washes from the cuticle of female conspecifics (Gaffin and Brownell, 1992). In addition, scorpions have sexually dimorphic chemosensory appendages called pectines (Kraeplin, 1907; Stahnke, 1974; Polis and Sissom, 1990; Gaffin and Brownell, 1997) that actively brush the substrate and become very active both before and during courtship (Alexander, 1957; 1959; Gaffin and Brownell, 1992; Tallarovic, 2000).

The role that chemical signals play in scorpion mate finding is not clear, although the reproductive behavior of desert scorpions suggests that mate trailing would be highly adaptive. Desert scorpions are solitary and nocturnal animals that mate for a few weeks during the late summer months when the temperatures are moderate. During this brief mating season, male scorpions are often found wandering dune habitats, presumably in search of receptive females (Polis and Farley, 1979; Polis, 1980). This wandering behavior increases male predation risk

(Polis and Farley, 1979; Polis and Sissom, 1990). Surface densities of scorpions vary during the reproductive season (4-40 individuals per ha, depending on species) but only a fraction of the animals on the surface are reproductive on a given night (Polis, 1990). Given the potentially hazardous and difficult nature of finding a receptive female, it seems implausible that undirected mate searching by the male could account for a significant number of reproductive encounters in desert scorpions.

We chose to investigate mate-trailing in scorpions using the giant hairy desert scorpion (*Hadrurus arizonensis*). This species is commonly found in the Mojave Desert of California and is the largest species of scorpion in North America. The animal is not easily disturbed or intimidated and will readily court and mate in the laboratory (Tallarovic, 2000). Reproductive activity can be artificially extended in captivity for a period of months (Tallarovic, 2000), and freshly collected giant hairy desert scorpions can be rapidly assayed (within minutes) for reproductive behavior (Melville et al., 1999; Tallarovic, 2000). More importantly, sex-specific courtship and conspecific aggressive behaviors have been well described and are easily discernable in this species (Tallarovic, 2000). The objectives of the present study were to determine the following: 1) are male scorpions capable of following the path of a receptive female, 2) is mate-trailing mediated by a sex-specific signal and 3) how is female substrate exposure related to trailing efficiency, locomotion, and male behavior. This was accomplished in three separate experiments using the Y-maze choice test.

Materials and Methods

Animals

Male and female giant hairy desert scorpions (*H. arizonensis*) were collected from the Mojave Desert in sand dune environments near Baker, Glamis and Indio, CA using portable black lights (Stahnke, 1972). Animals were collected during September 1998 for experiment #1 and August 1999 for experiment #2. All scorpions were sexed, given a collection number and housed separately in 16 oz. plastic containers with approximately 20 ml of native sand. Animals in experiments #1 and #2 were assayed for mating behavior immediately after capture and were used in Y-maze tests within 24 hours. Animals in experiment #3 were collected near Glamis and Indio CA and shipped to Oregon State University. Scorpions were housed in separate 16 oz plastic containers with 20 ml of heat treated sand, placed on a shifted light cycle (nightfall occurring at 4:30 p.m.) with daily heat cycles of 28-35°C, to mimic the natural dune environment in the east Mojave Desert (Brownell, unpublished data).

Apparatus

Behavioral Enclosure

Experiments in the field laboratory were conducted in a darkened room inside an enclosure (length = 150 cm, height = 75 cm, width = 75 cm). The enclosure was covered with black felt cloth on all sides and divided lengthwise into two identical sub-chambers using a black felt curtain (length = 75 cm, width = 75 cm). Each sub-chambers was illuminated by a red 25 watt bulb (distance = 50 cm) that scorpions cannot see (Machan, 1968). Air temperatures in the enclosure were within the seasonal range of late summer (30-35 °C) in the east Mojave Desert (Brownell per obs). In experiment #2, all tests were filmed from above using a low-light Watec® WAT-902A video camera. Behavioral tests performed at Oregon State University were conducted in a similar manner using a permanent behavioral enclosure (length = 2.4 m, height = 2.1 m, width = 1.2 m). As described above, the enclosure was divided into two identical sub-chambers. Each sub-chambers was illuminated by two red 25 watt bulbs (distance = 50 cm). In experiment #3, all tests were filmed from above as previously described. Air temperatures in the enclosure at Oregon State University were maintained between 28-32 °C with the aid of a floor heater (type 2507, Delonghi Inc.).

Y-mazes

Four identical Y-mazes, constructed from plexiglass and PVC tubing were used in this study (Fig 4.1). Y-maze design consisted of a circular starting arena (diameter = 23 cm, height = 15 cm), a main walkway and two maze arms. The main walkway of the Y-maze was 5 cm wide and 25 cm long. The maze arms were 15 cm long and 5 cm wide. The walls of the walkway and maze arms were 7.5 cm tall.

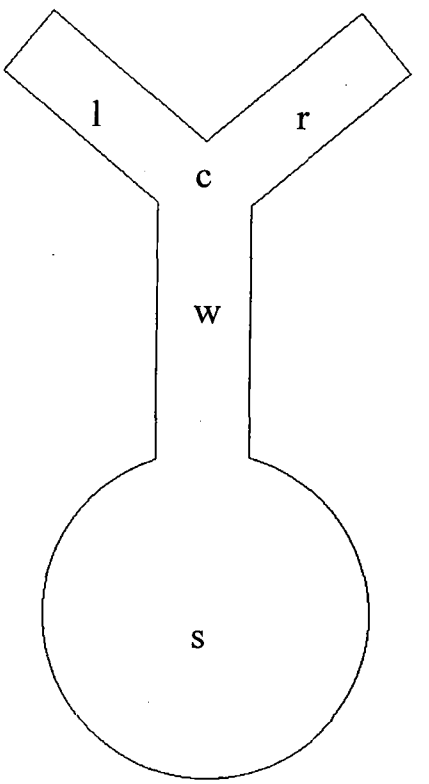


Figure 4.1 Y-Maze Design.
(A) The Y-maze consisted of a starting arena (s) and a main walkway (w) that split at the choice point (c) into the left (l) and right (r) arms of the Y maze.

Protocols

Mating Behavior Assay

We developed a behavioral assay to determine if male and female scorpions were reproductively active. Male and female scorpions were randomly paired and tested in a circular PVC arena (diameter = 23 cm, height = 15 cm) filled with native sand. Only animals that exhibited described courtship behaviors for this species including the *Lunge/Sting* and *Promenade-a-deux* (Tallarovic, 2000) were used in Y-maze tests. Courtship was terminated by gently dripping water on courting pairs. This induced rapid separation from the *promenade* without injury to either animal. Procedures for courtship termination did not adversely affect mating receptivity. Scorpions were re-assayed against different individuals and expressed courtship behaviors.

Y-maze Test Protocol

Two Y-maze tests were performed simultaneously in each sub-chamber of the enclosure. Before each test, the Y-mazes and their lane dividers were cleaned with 70% ethanol, rinsed with distilled water, wiped clean with paper towels and allowed to air dry. The Y-mazes were used in sequential order (#1-4). During each test the Y-maze was placed in a randomly chosen sub-chamber with the maze arms

placed in one of four randomly chosen directions (north, south, east, or west). Each Y-maze was then filled with native sand to a depth of 1.5 cm to form a smooth and even substrate.

During the course of an experiment, each male scorpion was tested in one experimental and one control test. In the first experiment, the control tests were conducted before the experimental tests. In the second and third experiments, experimental and control tests were conducted simultaneously in a randomized order. Male scorpions were tested once per night. In both experimental and control tests, all animals were tested systematically in random order. In experimental tests, the direction of the 'trail' left by the 'labeling' animal was determined by the flip of a coin (heads = left, tails = right). The opposite arm was blocked using a plastic divider and the 'labeling' animal was allowed to walk down the Y-maze once. The scorpion and the plastic divider were then removed from the Y-maze and placed outside the behavioral enclosure. The male was then placed in the starting arena, opposite the opening of the walkway. Negative controls were performed in the same manner by excluding the labeling animal. Maze arm choice was recorded when the male touched the end of one of the maze arms. If movement was not observed after a period of 15 minutes the test was terminated and not included in the analysis. Individuals that climbed out of the Y-maze during the test were also excluded from the analysis. The sand was then removed from the Y-maze and the Y-maze was then cleaned as described and prepared for another test.

Experiments

Trailing in Scorpions with Mate-pairing (Exp. #1)

In this first experiment, the Y-maze choice test was performed to investigate if male scorpions could trail female conspecifics. To maximize male receptivity, we tested scorpions in reproductive pairs. A reproductive pair was classified as two animals (male and female) that initiated courtship and proceeded to the *promenade* in the mating behavior assay. Ten reproductive pairs were used in this experiment. Females were only allowed to walk down the Y-maze a single time in experimental tests. This experiment was conducted in September 1998 from 18:00-23:00 hr near Baker, CA at the Zzyzx Desert Studies Center, maintained by California State University, Fullerton.

Sex-specific Trailing in Scorpions Without Mate-pairing (Exp. #2)

A second experiment was conducted to investigate if male scorpions were capable of trailing female conspecifics without mate pairing and to determine if male trailing required a sex-specific signal. Twenty-nine male scorpions were each tested in a Y-maze against a trail left by one of 5 randomly chosen female scorpions. All scorpions used were shown to be reproductively active using the mating behavior assay. Control and experimental tests were conducted simultaneously and recorded on T160 videotape. Positive controls were conducted

in the same fashion after the completion of experimental and control tests. Each male scorpion was tested against the trail left by one of 5 other randomly chosen male scorpions. In this experiment, all Y-maze tests were conducted in August 1999 from 21:00– 04:00 hr. at the Desert Studies Center.

Effect of Increased Substrate Exposure on Trailing, Locomotor Pattern and Behavior of Male Scorpions (Exp. #3)

To determine what effect an increase in female substrate exposure would have on trailing efficiency, locomotion, and male behavior, a third experiment was conducted. Twenty reproductive male scorpions were assayed against a trail left by one of 5 female scorpions chosen at random. The females used in this test were shown to be the most desirable in a previous mate-choice study (Tallarovic, 2000). The Y-maze protocol was changed in two ways. Female scorpions were allowed to walk up and down the Y-maze five times, and the Y-mazes were placed in large plastic containers (length = 71 cm, width = 43 cm, height = 22 cm) to reduce the possibility of escape. Tests were conducted in November 1998 from 19:00-24:00 hr at Oregon State University. Control and experimental tests were recorded on T160 videotape. A researcher blind to treatment type scored each recorded test for evidence of pre-courtship behavior, lane choice, and test duration.

One researcher conducted behavioral analysis of locomotor pattern in recorded experimental and control tests. A 5-volt pulser was attached to the input

channel of a chart recorder (Linear inc.). Amplifier settings were 1 volt / cm for the vertical axis and the recording speed was set at 30 cm / minute. The duration of movements in experimental and control tests were recorded by holding the pulser high when an animal moved. When movement ceased, the pulser was released. As shown in figure 4.2, male scorpions moved in bouts that consisted of a series of sprints followed by intermittent pauses. A bout was classified as a group of sprints occurring in short succession (within two seconds of each other). A pause was classified as the interval between two bouts (greater than 2 seconds). The average number and duration of sprints, bouts and pauses were measured for each control and experimental test.

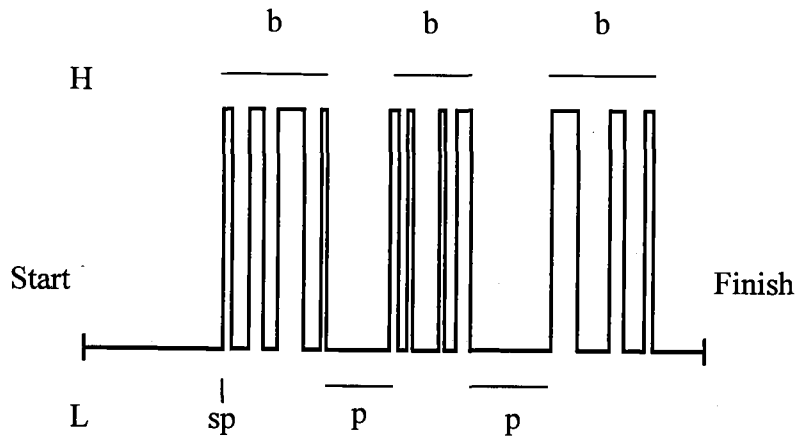


Figure 4.2 Example record of scorpion movement.
When the scorpion moved the pulser was held high (H) and when movement stopped the pulser was released (L). This produced a linear record from the start to the end of the trial. Scorpions moved in characteristic bouts (b). Each bout is composed of shorter sprints (sp). Between each bout the animal has paused (p).

To determine if the results from experiment #3 were sex specific, we repeated this experiment at a later date substituting 'labeling' males for females.

This experiment was conducted in August 1999 from 21:00– 04:00 hr at the Desert Studies Center. Twenty-nine male scorpions were tested in the Y-maze against a trail left by one of 5 other male conspecifics chosen at random. The results from experiment #2 served as the negative controls. All scorpions used were shown to be reproductively active using the mate behavior assay. Male scorpions were tested once per night. The 'labeling' male was allowed to walk up and down the Y-maze five times, mimicking the conditions of the female tests. In this experiment, Y-mazes were not placed in plastic containers during the tests.

Statistical Analysis

The binomial distribution was used to determine if male scorpions were biased to either arm of the Y-maze in control tests and to determine if male scorpions preferred maze arms exposed to female or male conspecifics (exp. 1-3). Scorpion locomotor pattern in control and experimental tests were compared using the students' t-test (exp. 3). A chi-square test for lack of independence was performed to determine if behaviors elicited in experimental tests (substrate exposed to female) were significantly different from negative (blank substrate) and positive (substrate exposed to another male) controls (exp. 3). Statistical analyses were completed using STATGRAPHICS Plus for Windows (v4, Statistical Graphics Corp.).

Results

When placed in the starting arena, male scorpions exhibited a brief period of quiescence (< 5 min) and then actively explored the Y-maze. Normal climbing, digging, and grooming behaviors were exhibited in all trials.

Trailing with reproductive pairs

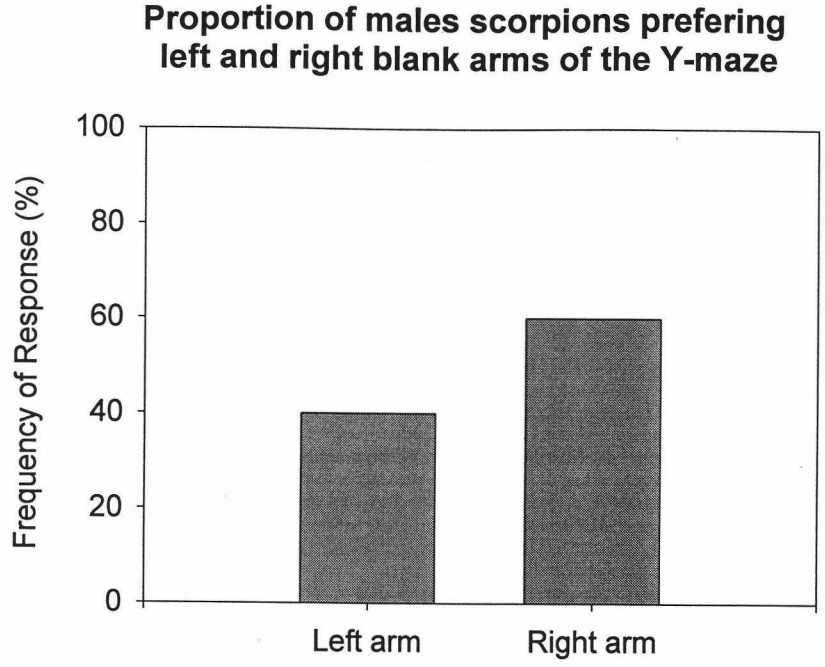
No bias was observed for either arm of the Y-maze in control tests ($n = 10$, $p > 0.05$). A significant proportion of male scorpions preferred the arm of the maze that the female scorpion had walked down ($n = 10$, $p < 0.01$) (Fig 4.3).

Trailing in scorpions without mate-pairing

A significant proportion of male scorpions, without previous courting, preferred the arm of the Y-maze exposed to a reproductive female ($n = 29$, $p < 0.037$). Male scorpions did not prefer Y-maze arms exposed to other male conspecifics ($n = 27$, $p > 0.05$) (Fig 4.4). No bias was observed in the negative controls. Forty-eight percent of the male scorpions chose the left arm and 52% chose the right arm of the Y-maze when the arms were blank ($n = 26$, $p > 0.05$).

Figure 4.3 Evidence of mate-trailing with receptive pairs.
(A) Proportion of male scorpions that walked down the left and right arms of the Y-maze in the control tests ($n = 10$, $p > 0.05$). (B) Proportion of male scorpions that walked down the arm of the Y-maze exposed to a female conspecific ($n = 10$, $p < 0.01$). Asterisk denotes significant departure ($p < 0.05$) from 50% using the binomial distribution.

A


Proportion of males scorpions prefering left and right blank arms of the Y-maze
Frequency of Response (%)
100
80
60
40
20
0
Left arm
Right arm

B

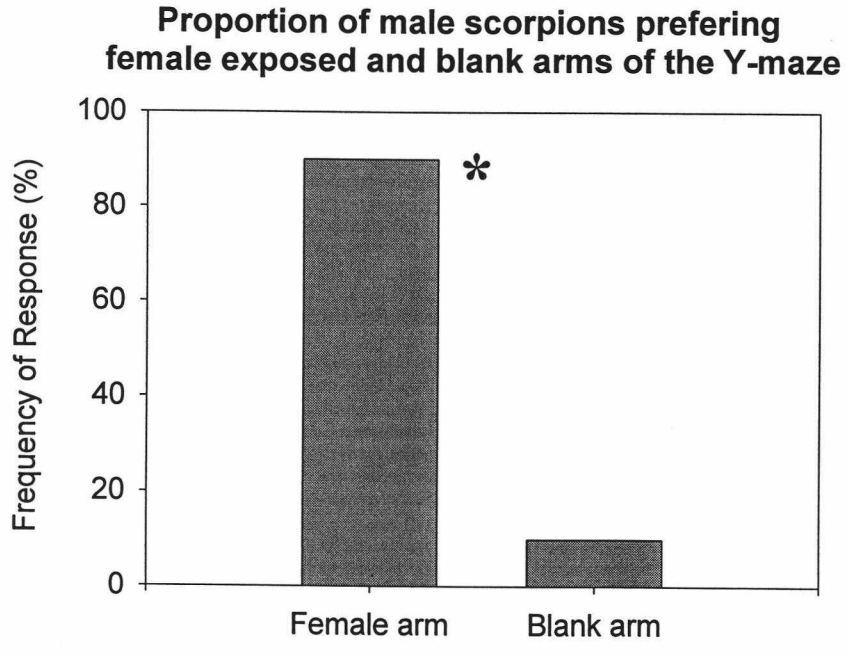
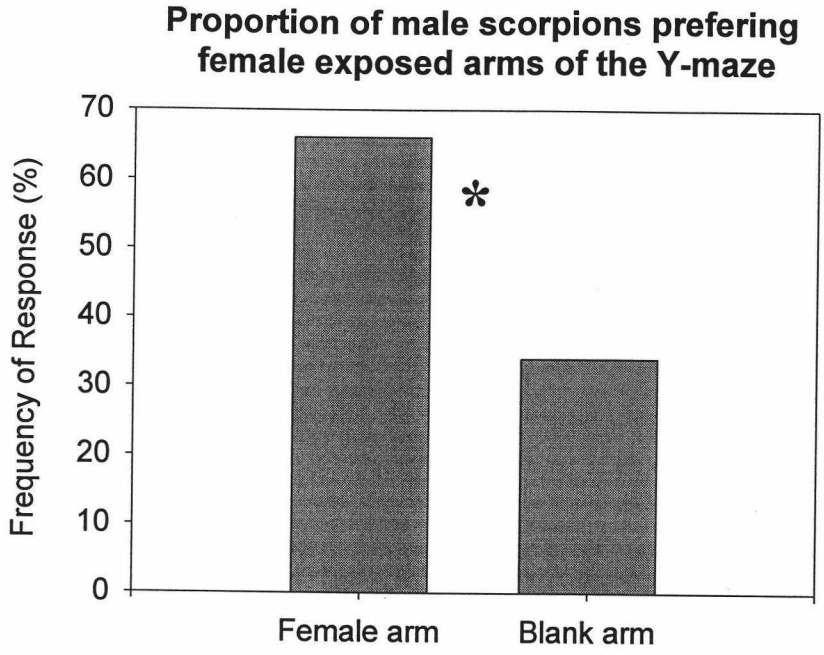
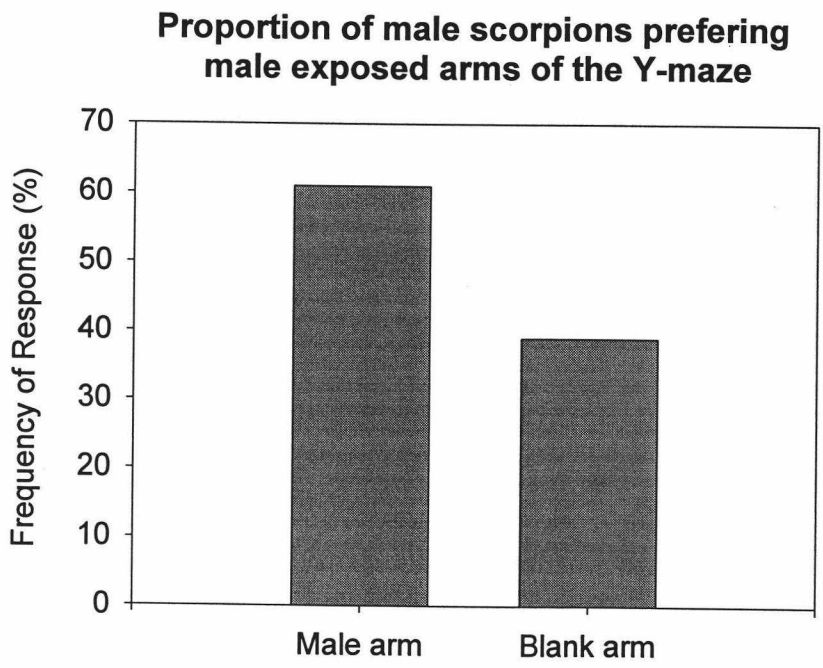

Proportion of male scorpions prefering female exposed and blank arms of the Y-maze
Frequency of Response (%)
100
80
60
40
20
0
*
Female arm
Blank arm

Figure 4.4 Evidence of sex-specific trailing in male scorpions.
(A) Proportion of male scorpions that walked down the arm of the Y-maze exposed to a female conspecific ($n = 29$, $p < 0.037$). (B) Proportion of male scorpions that walked down the arm of the Y-maze exposed to a male conspecific ($n = 27$, $p > 0.05$). Asterisk denotes significant departure ($p < 0.05$) from 50% using the binomial distribution.

A


Proportion of male scorpions prefering female exposed arms of the Y-maze
Frequency of Response (%)
70
60
50
40
30
20
10
0
*
Female arm
Blank arm

B


Proportion of male scorpions prefering male exposed arms of the Y-maze
Frequency of Response (%)
70
60
50
40
30
20
10
0
Male arm
Blank arm

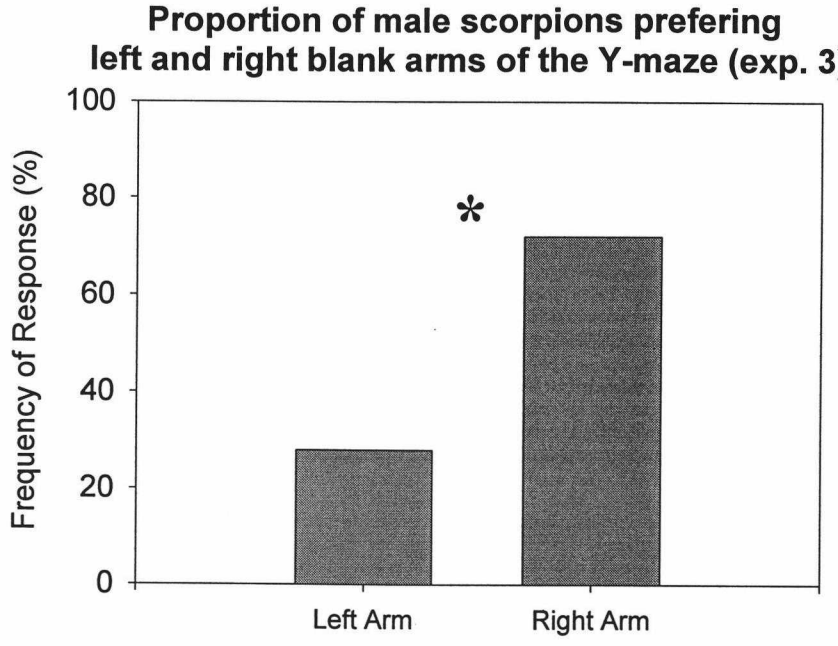
Effect of increased substrate exposure on trailing, locomotor pattern and behavior of male scorpions

Y-maze test results

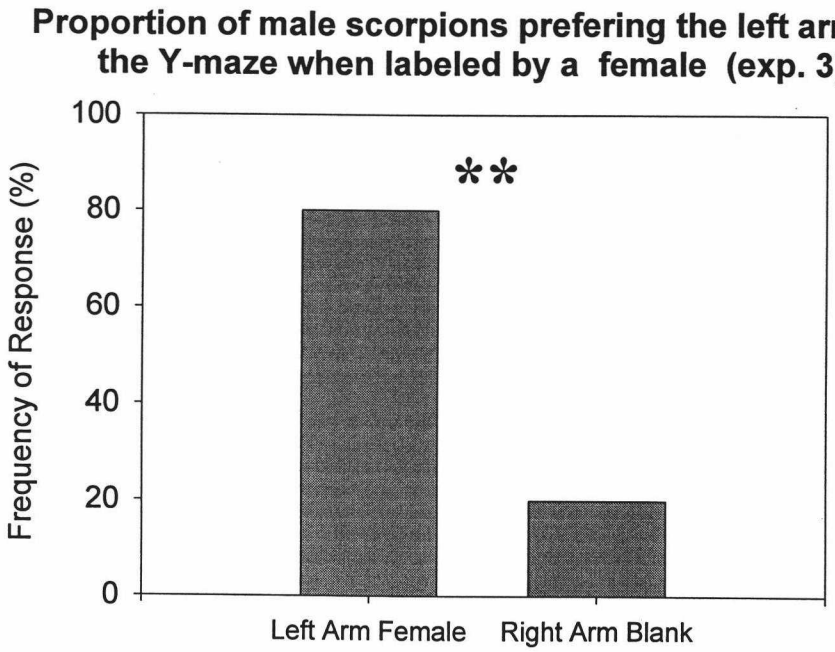
A significant proportion of male scorpions preferred the arm of the Y-maze after it had been exposed to a female 5 times. Seventy four percent of males tested chose the arm that the female had walked down. This proportion would have been statistically significant ($n = 19$, $p < 0.022$) if there was no bias in the controls. Unlike the two other tests, a right arm bias was found in the controls. Seventy two percent of male scorpions in the control tests chose the right arm ($n = 18$, $p < 0.05$). Given this right arm preference of the males, few males should prefer the left arm of the Y-maze even after a female has walked down it, unless male scorpions are trailing female conspecifics. This is a plausible hypothesis. A significant proportion of male scorpions preferred the left arm of the Y-maze when it was exposed to a female (Fig 4.5). It is highly unlikely that this response was due to chance ($X^2 = 7.05$, $p < 0.01$, $n = 10$). The bias present in the controls was likely due to a reversible change in protocol. We found that Y-mazes placed in plastic containers leaned to the right, creating a downward slope of approximately 5 degrees. This was the likely source of the bias observed. No bias was detected in control tests conducted when plastic containers were not used during the Y-maze test ($n = 100$ after combining control tests conducted prior (exp. 1) and after (exp. 2) this experiment).

Figure 4.5 Effect of increased female substrate exposure on mate-trailing in scorpions.
(A) Proportion of male scorpions walking down the left and right arms of the Y-maze test during control (blank) tests at Oregon State University (n = 18). (B) Proportion of male scorpions that walked down the left arm of the Y-maze when the female walked down the left arm. Asterisk denotes significant departure ($p < 0.05$) from 50% using the binomial distribution. Double asterisk denotes proportions observed in B are significantly different from A using the chi-square test for lack of independence ($X^2 = 7.05$, $p < 0.01$, $n = 10$).

A


Proportion of male scorpions prefering left and right blank arms of the Y-maze (exp. 3)
Frequency of Response (%)
100
80
60
40
20
0
*
Left Arm
Right Arm

B


Proportion of male scorpions prefering the left arm of the Y-maze when labeled by a female (exp. 3)
Frequency of Response (%)
100
80
60
40
20
0
**
Left Arm Female
Right Arm Blank

Analysis of Locomotor Pattern

The locomotor pattern of male *H. arizonensis* was altered when the substrate was exposed to a reproductive female. Experimental tests (female exposed substrate) were significantly longer than control (blank substrate) tests (t-stat -2.16, $p < 0.04$). The average test duration was 6.81 ($\pm$9.77, n = 19) and 1.75 ($\pm$1.74, n = 18) minutes for experimental and control Y-maze tests respectively. In addition, male scorpions exhibited significantly more pauses in experimental tests than in control tests (t-stat -2.65, $p < 0.01$). There were 6.71 ($\pm$5.27, n = 14) pauses on average in experimental tests compared to 2.74 ($\pm$2.05, n = 14) pauses on average in controls. All of the remaining ambulatory parameters were greater on average when the substrate was exposed to a female, but were not statistically different from controls (table 4.1).

Table 4.1 Comparison of measured locomotor parameters in experimental (female exposed substrate) and control (blank substrate) Y-maze tests.

	Control (blank)			Exp. (Female)			*t*-test	
Parameter	Average	std	n	verage	std	n	t-stat	p-value
Bout duration (sec)	11.68	6.83	16	16.17	14.13	19	-1.16	< 0.25
Bout number	3.23	2.19	16	5.36	5.12	19	-1.59	< 0.12
Sprint duration (sec)	0.88	0.32	16	1.10	0.42	19	-1.76	< 0.08
Sprint number	0.87	0.32	16	1.09	0.42	19	-1.73	< 0.09
Pause duration	12.44	8.46	14	17.92	19.66	14	-0.96	< 0.35
Pause number	2.74	2.05	14	6.71	5.27	14	-2.65	< 0.01*

Asterisk denotes statistically significant difference ($p < 0.05$) from control values.
(std = standard deviation, n = number of trials, *t*-stat = students' *t*-test statistic)

Behaviors Observed

Three discrete behaviors were evoked in male *H. arizonensis* when they encountered substrates exposed to a reproductive female in the Y-maze test. Two of these behaviors resembled 'juddering' and 'tail-wagging' responses that have been described as pre-courtship behaviors in other species of scorpions (Gaffin and Brownell, 1992). Juddering involved slow rocking or rapid pulses of the main body. Tail-wagging involved shaking of the last metasomal segment up and down or across the body axis at frequencies above 4 hz. The third response resembled mate-seizing behavior as described in *H. arizonensis* and other species of scorpions (Polis and Farley, 1979; Tallarovic, 2000) and only occurred after a male made contact with a wall or corner that was previously touched by a reproductive female. We described this novel behavior as 'lunging'. During a 'lunge', the metasoma was arched over the body as the scorpion sprinted or turned into the object that had been touched by the female. The male would then forcefully attack the object with both the pedipalps and aculeus.

The behaviors described were not evoked in negative (blank) controls. When males encountered substrates exposed to male conspecifics, juddering was observed, but in only 1 of the 25 tests. Tail-wagging and lunging were not evoked. When the substrate was exposed to a female, over 60 percent of male *H. arizonensis* exhibited one or more of the behaviors described. More than 50% of the males exhibited tail-wagging, more than 40% exhibited lunging behavior and more than 25% exhibited juddering. Males exhibited significantly more of all of

the behaviors described when the substrate was exposed to a reproductive female (table 4.2)($X^2 = 282$, df = 3, $p < 0.005$). Several of these behaviors were often released during the same experimental test (table 4.3).

Table 4.2 Proportion of behaviors evoked in male scorpions after contacting substrates exposed to female and male conspecifics and untreated substrates.

	Proportion of Males that Displayed Pre-Courtship Behavior to		
Behavior	Female exp. substrate	Male exp. substrate	Blank Control
Judder	0.26	0.04	0
Tailwag	0.52	0	0
Lunge	0.47	0	0
Combined	0.63	0	0
Number of Trials	19	25	18

Table 4.3 Behaviors elicited in the same test when the substrate was exposed to a female or male conspecific.

	Proportion of Behaviors Elicited Alone and Together	
Behaviors Elicited	Female exp. substrate	Male exp. Substrate
Judder	0	1
Tailwag	0.25	0
Lunge	0	0
Judder and Lunge	0.17	0
Judder and Tailwag	0.08	0
Tailwag and Lunge	0.33	0
All Three Behaviors	0.17	0

The values listed are from tests that elicited a behavioral response.
(n = 12 for female-labeled tests, n = 1 for male-labeled tests)

Discussion

The results from this study suggest that male scorpions can orient and respond to substrate borne signals from female conspecifics. In all three experiments, a significant proportion of male scorpions preferred the arm of the maze that a reproductive female had walked down. Male scorpions did not prefer maze arms that male conspecifics had walked down, indicating that this response was sex-specific. When the substrate was repeatedly exposed to a reproductive female, males took significantly longer to complete the Y-maze, exhibited an increase in pausing behavior, and displayed juddering, tail-wagging, and lunging responses resembling pre-courtship behavior in *H. arizonensis* and other desert scorpions (Gaffin and Brownell, 1992; Tallarovic, 2000). These behaviors were not elicited in negative controls (untreated sand) and although juddering was elicited in positive controls (substrate exposed to male conspecific) it occurred in only 1 of 25 trials (< 5%). In contrast, tail-wagging and lunging were only elicited when the substrate was exposed to a female conspecific.

During the mating season, male desert scorpions must travel a considerable distance to find a receptive mate. Random wandering by the male might produce a significant number of female encounters for a species with high surface densities (*P. mesaensis* = 30 to 40 animals per ha)(Polis, 1990), but this is not the case with *H. arizonensis* (only 4 animals per ha)(Polis, 1990). The number of giant hairy desert scorpions commonly found on the surface is an order of magnitude lower

than *P. mesaensis*, suggesting that some form of sexual communication may be required for mate finding. The results from the Y-maze tests suggest that male *H. arizonensis* can follow the trails of reproductive females and may use substrate borne chemical cues for this purpose.

Trailing behavior in *H. arizonensis* appears to require a female signal. In all three tests, male *H. arizonensis* preferred the arm of the Y-maze that a reproductive female had walked down. This preference appears sex-specific, as male scorpions did not show a significant preference for the arm of the Y-maze that a male scorpion had walked down. The simplest explanation for this sex-specific preference is that female scorpions deposit sex pheromones on the substrate as they walk. Support for this hypothesis comes from previous behavioral studies in the desert sand scorpion (*P. mesaensis*). Male desert scorpions were placed in a circular arena containing four quadrants. The sand in one of the quadrants was exposed to a female or treated with hexane washes from the cuticle of female conspecifics. When male scorpions contacted treated quadrants, turning behaviors were elicited in the male that led to an increase in occupancy of the treated area (Gaffin and Brownell, 1992). The present study adds to this observation by demonstrating that male giant hairy desert scorpions can discriminate and orient to sex-specific signals that are likely chemical in origin.

When the substrate was repeatedly exposed to a reproductive female, males took significantly longer to complete the Y-maze and exhibited an increase in pausing behavior. The function of pausing is not known, but in the experiment

described above, Gaffin and Brownell, (1992) observed that male desert scorpions increase pectinal tapping frequency and spent more time in female treated quadrants. This suggests that the increase in test duration and pause number observed in the present study may be associated with mate searching behaviors in *H. arizonensis* and increased substrate sampling by the chemosensory pectines.

Juddering, tail-wagging and a novel behavior described as lunging were elicited in male scorpions when they contacted substrates that had been previously occupied by a reproductive female. Juddering is assumed to be a pre-courtship behavior that signals the female by producing substrate vibrations (Alexander, 1957; Rosin and Shulov, 1963; Polis and Farley, 1979), but it is not known if this response is limited to mating encounters. In the case of *H. arizonensis*, juddering occurs in all conspecific interactions (Tallarovic, 2000) and therefore cannot be classified solely as a pre-courtship behavior in this species. In contrast, tail wagging has never been observed in interactions involving two males (Tallarovic, 2000) and likely represents pre-courtship signaling in *H. arizonensis* and other scorpions (Gaffin and Brownell, 1992). Of particular relevance was male lunging. This behavior resembled mate-seizing behavior, a stereotypic response only observed during the first phase of courtship in *H. arizonensis* (Tallarovic, 2000). Lunging was only observed after the male made contact with a wall or corner that the female had previously touched, suggesting that a sex-specific signal deposited on the walls of the Y-maze was capable of releasing mate-seizing behavior.

Sex specific signals from female scorpions apparently serve as both male attractants and courtship 'releasers', but at different levels of exposure. In all of the tests, a significant proportion of male scorpions preferred the arm of the Y-maze that a conspecific female had walked down, but pre-courtship behaviors were only elicited when the substrate had been exposed to a female at least 5 times. This differential response suggests that at low levels, a sex-specific signal may orient the male to the female, and that at higher levels, the signal may release pre-courtship behavior in male *H. arizonensis*.

Chemical communication in scorpions and spiders may be very similar. Spiders utilize both volatile (Schultz and Toft, 1993) and contact sex pheromones (Hegdekar and Dondale, 1969; Suter and Renkes, 1982; Trabalon et al., 1997). The sex pheromones are bound to the silk (Roland, 1984; Suter and Hirscheimer, 1986) and possibly the cuticle of female conspecifics (Suter et al., 1987; Trabalon et al., 1997; Prouvost et al., 1999), serving as both male attractants and courtship 'releasers' (Suter and Renkes, 1982, Jackson, 1987). Male spiders will actively trail female drag-lines (Tietjen, 1977; Tietjen and Rovner, 1980; Taylor, 1998) and exhibit courtship behaviors after contacting both female webs and substrates previously occupied by females (Jackson, 1987; Barth and Schmitt, 1991; Prouvost et al., 1999).

The probability of finding and isolating sex pheromones is greatly enhanced with the use of behavioral assays (Mason, 1993). Mate-signaling in the desert sand scorpion (*P. mesaensis*) appears to be mediated by sex pheromones that are

cuticular in origin (Gaffin and Brownell, 1992), but the development of a behavioral assay to screen cuticular extracts has been problematic using this species (response rate < 20%) (Gaffin, pers obs). We have developed a behavioral model to study scorpion mate-signaling using *H. arizonensis*. Male *H. arizonensis* will follow the trail of female conspecifics in the Y-maze test (response rate > 60%), and produce robust pre-courtship behaviors (response rate > 60%) when they contact sand previously occupied by a reproductive female. The Y-maze test, or a behavioral assay using the pre-courtship behaviors described, can now be used to screen sex-specific differences in lipid profiles in *H. arizonensis* (Thevenieau, 1999) that could serve as sex pheromones.

It is not known how male scorpions detect sex pheromones from the female, but both the pectines and pedipalps of scorpions have chemosensory sensilla that may be involved. The pectines are paired, comb-like appendages that project ventrally from the 2nd mesosomal segment of all scorpions (Hjelle, 1990). Each pectine supports thousands of chemosensory sensilla called pegs (Swoveland, 1978; Foelix and Muller-Vorholt, 1983; Gaffin and Brownell, 1997) that are actively swept across the surface both before and during courtship (Alexander, 1957; 1959; Gaffin and Brownell, 1992; Tallarovic, 2000). Male scorpions also increase pectinal sweeping activity when they contact sand treated with chemical extracts from the cuticle of female conspecifics (Gaffin and Brownell, 1992). This observation suggests that the pectines may be used in pheromone detection. In this study, scorpions explored the starting arena, main walkway, and choice points of

the Y-maze using their pedipalps. The tarsi and pedipalps of scorpions support sensilla that resemble chemosensory hairs in arachnids (Venkateswara, 1963; Foelix and Schabranath, 1983; see Root, 1990 for review) but they have not been adequately studied. With the development of *H. arizonensis* as a behavioral model to study pheromonal signaling in scorpions, a series of trailing experiments using sensory blocking methods could readily resolve this question.

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References

Abelson PH. 1985. Use of and research on pheromones. *Science* 229:1342.

Alexander AJ. 1957. The courtship and mating of the scorpion *Opisthophthalmus latimanus*. *Proc Zool Soc Lond* 128:529-44.

Alexander AJ. 1959. Courtship and mating in the buthid scorpions. *Proc Zool Soc Lond* 133:145-169.

Barth FG. 1985. Neuroethology of the spider vibration sense. In: Barth FG, editor. *Neurobiology of Arachnids*. New York: Springer-Verlag. pp 203-229.

Barth FG, Schmitt A. 1991. Species recognition and species isolation in wandering spiders (*Cupiennius* sp: Ctenidae). *Behav Ecol Sociobiol* 29:333-339.

Bell WJ, Kipp LR, Collins RD. 1995. The role of chemo-orientation in search behavior. In: Carde RT, Bell WJ, editors. *Chemical Ecology of Insects 2*, New York: Chapman and Hall. pp 105-153.

Berger RS. 1972. 2,6-Dichlorophenol, sex pheromone of the lone star tick. *Science* 177:704-705.

Carde RT, TC Baker. 1984. Sexual communication with pheromones. In: Bell W, Carde RT, editors. *Chemical Ecology of Insects*. London: Chapman and Hall. pp 355-376.

Dusenberry DB. 1993. *Sensory ecology: how organisms acquire and respond to information*. New York: WH Freeman and Company.

Dryer FC, Brockman JH. 1996. Sensory processes, orientation, and communication: biology of the Umwelt. In: Houck LD, Drickamer LC, editors. *Foundations of animal behavior: classic papers with commentaries*. Chicago: Univ Chicago Press. pp. 529-538.

Ewing AW. 1989. *Arthropod bioacoustics: neurobiology and behavior*. New York: Cornell Univ Press.

Foelix RF, Muller-Vorholt G. 1983. The fine structure of scorpion sensory organs. II. Pectine sensilla. *Bull Brit Arachnol Soc* 6:68-74.

Foelix RF, Schabranath J. 1983. The fine structure of scorpion sensory organs. I. Tarsal sensilla. *Bull Brit Arachnol Soc* 6:53-67.

Gaffin DD, Brownell PH. 1992. Evidence of chemical signaling in the sand scorpion, *Paruroctonus mesaensis* (Scorpionida: Vaejovidae). *Ethology* 91:59-69.

Gaffin DD, Brownell PH. 1997. Response properties of chemosensory peg sensilla on the pectines of scorpiones. *J Comp Physiol (A)* 181:291-300.

Gaffin DD, Brownell PH. 2000. Chemosensory behavior and physiology. In: Brownell PH, Polis G, editors. *Scorpion Biology and Research.* New York: Oxford Univ Press. pp 223-245.

Hegdekar BM, Dondale CD. 1969. A contact sex pheromone and response parameters in Lycosid spiders. *Can J Zool* 47:1-4.

Hjelle JT. 1990. Anatomy and morphology. In: Polis GA, editor. *The biology of scorpions*. Stanford: Stanford Univ Press. pp 9-63.

Jackson PR. 1987. An analysis of alternative mating tactics of the jumping spider, *Phidippus johnsoni* (Araneae, Salticidae). *J Arachnol* 5:185-230.

Kraeplin K. 1907. Die sekundaren geschlechtscharaktere der scorpion, pediplapen und solifugen. *Mitteilungen aus dem Naturhistorichen Museum in Hamburg* 25:185-225.

Krapf D. 1986. Contact chemoreception of prey in hunting scorpions (Arachnida: Scorpiones). *Zool Anz* 217:119-129.

Lewis DB, Gower DM. 1980. *Biology of Communication.* New York: Wiley-Liss.

Lloyd JE. 1983. Bioluminescence and communication in insects. *Annu Rev Entomol* 28:131-160.

Machan L. 1968. Spectral sensitivity of scorpion eyes and the possible role of shielding pigment effect. *J Exp Biol* 49:95-105.

Mason RT. 1993. Chemical ecology of the red-sided garter snake, *Thamnophis sirtalis parietalis. Brain Behav Evol.* 41:261-268.

Polis GA, Farley RD. 1979. Behavior and ecology of mating in the cannibalistic scorpion *Paruroctonus mesaensis* (Stahnke) (Scorpionida:Vaejovidae). *J Arachnol* 7:33-46.

Polis GA. 1980. Seasonal patterns and age-specific variation in the surface activity of a population of desert scorpions in relation to environmental factors. *J Anim Ecol* 49:1-18.

Polis GA. 1990. Ecology. In: Polis GA, editor. *The biology of scorpions*. Stanford: Stanford Univ Press. pp 161-223.

Polis GA, Sissom WD. 1990. Life history. In: Polis GA, editor. *The biology of scorpions*. Stanford: Stanford Univ Press. pp 161-223.

Prouvost O, Trabalon M, Papke M, Schulz S. 1999. Contact sex signals on web and cuticle of *Tegenaria atrica* (Araneae, Agelenidae). *Arch Insect Biochem* Physiol 40:194-202.

Pollard SD, Macnab AM, Jackson RR. 1987. Communication with chemicals: pheromones and spiders. In: Nentwig W, editor. *Ecophysiology of spiders*. New York: Springer-Verlag. pp 133-141.

Roland C. 1984. Chemical signals bound to the silk in spider communication (Arachnida, Araneae). *J Arachnol* 11:309-314.

Root TH. 1990. Neurobiology. In: Polis GA, editor. *The biology of scorpions*. Stanford: Stanford Univ Press. pp 341-409.

Rosin R, Shulov A. 1963. Studies on the scorpion *Nebo hierichonticus*. *Proc Zool Soc Lond* 140:547-75.

Rovner JS, Barth FG. 1981. Vibratory communication through living plants by a tropical wandering spider. *Science* 214:464-466.

Schultz S, Toft S. 1993. Identification of a sex pheromone from a spider. *Science* 260:1635-1637.

Schultz S. 1997. The chemistry of spider toxins and spider silk. *Angew Chem Int Ed Engl* 36:314-326.

Sissom D. 1990. Systematics, Biogeography and Paleontology, In: Polis GA, editor. *The biology of scorpions*. Stanford: Stanford Univ Press. pp 161-223.

Sonenshine DE. 1985. Pheromones and other semiochemicals of the Acari. *Annu Rev Entomol* 30:1-28.

Stahnke HL. 1972. UV light, a useful field tool. *Bioscience* 22:604-607.

Stahnke HL. 1974. Revision and keys to higher categories of Vaejovidae. J *Arachnol* 1:107-141

Suter RB, Renkes G. 1982. Linyphiid spider courtship: releaser and attractant functions of a contact sex pheromone. *Anim Behav* 30:714-718.

Suter RB, Hirscheimer AJ. 1986. Multiple web-borne pheromones in a spider *Frontinella pyramitella* (Araneae: Linyphiidae). *Anim Behav* 34:748-753.

Suter RB, Shane CM, Hirscheimer AJ. 1987. Communication by cuticular pheromones in a Linyphiid spider. *J Arachnol* 15:157-162.

Swoveland MC. 1978. External morphology of the scorpion pectines. Masters thesis, California State University, San Francisco.

Tallarovic SK. 2000. Conspecific and mating behaviors of the giant hairy desert scorpion, Ph.D. Dissertation, Oregon State University, Corvallis.

Taylor PW. 1998. Dragline-mediated mate searching in *Trite planiceps* (Araneae, Salticidae). *J Arachnol* 26:330-334.

Thevenieau Laurent. 1999. Rapport de stage pheromones de contact. Maitrise de biologie cellulaire et de physiologie, Universite de la Mediterranee, Aix Marseille II.

Tietjen WJ. 1977. Dragline-following by male lycosid spiders. *Psyche* 84:165-178.

Tietjen WJ, Rovner JS. 1980. Trail-following behavior in two species of wolf spiders: sensory and etho-ecological concomitants. *Anim Behav.* 28:735-741.

Tietjen WJ, Rovner JS. 1982. Chemical communication in lycosids and other spiders. In: Witt PN, Rovner JS, editors. *Spider communication: mechanisms and ecological significance*. Princeton: Princeton Univ Press. pp 249-280.

Trabalon M, Bagneres AG, Roland C. 1997. Contact sex signals in two sympatric spider species, *Tegenaria domestica* and *Tegenaria pagana*. *J Chem Ecol* 23:747-758.

Venkateswara RP. 1963. Studies on the peripheral nervous system of the scorpion, *Heterometrus fulvipes*. Ph.D. Dissertation, Sri Venkateswara University, Tirupati, Andhra Pradesh, India.

Walker TJ. 1957. Specificity in the response of female tree crickets (Orthoptera, Gryllidae, Oecanthinae) to calling songs of the males. *Ann Entomol Soc Am* 50:626-636.

Warburton C. 1909. Arachnida embolobranchiata: Scorpions, spiders, mites, etc. In: Harmer SF, Shipley AE, editors. *The Cambridge Natural History. V4 Crustacea and Arachnida*. London: Macmillan press. pp 295-473.

Chapter 5: Conclusion

This body of work contains two anatomical studies that describe the peripheral and central organization of the pectines, the primary chemosensory system of scorpions. A third study demonstrates experimentally that male scorpions are capable of following the paths of female conspecifics. The results from the anatomical and behavioral studies are summarized and their implications are discussed.

Peripheral Organization of the Pectinal Teeth

Three cytoarchitectural layers were located beneath the peg sensilla: a layer of sensory dendrites, a neuronal cell layer and an axonal plexiform layer. The neuronal cell layer was further divided into two layers of small and large neuronal somata defined as the outer and inner nuclear layers (ONL, INL). The dense fields of peg sensilla on the pectinal teeth were innervated by cassettes of sensory neurons organized in orderly vertical columns orthogonal to the horizontal layers described. Each sensillar cassette contained a regular complement of dendrites originating from the two classes of neurons in the ONL and INL. The two cell types in each cassette may correspond to separate chemosensory and mechanosensory neurons that innervate the peg sensilla. The axonal layer below the two nuclear layers was immunoreactive to antibodies against the synaptic vesicle protein Synapsin, revealing small clusters of fluorescent labeling. Numerous axonal swellings were

observed between sensory axons and were classified into three main categories based on examples from Golgi preparations.

Each pectinal tooth may detect surface textures and substrate chemistry with topographic precision, a function analogous to a 'chemotactic' retina. Evidence supporting this hypothesis comes from both current and previous studies that have investigated the structure and function of the peg sensilla on the pectinal teeth. The ventral surface of the teeth supports a dense field of sensilla innervated by parallel cassettes of sensory neurons that respond to both chemical and mechanical stimuli (Gaffin and Brownell, 1997a; Melville and Brownell, 1997). The sensory cassettes are organized horizontally into discrete laminae of dendrites, cell bodies and an axonal layer of peripheral synapses (Foelix and Muller-Vorholt, 1983; Melville and Brownell, 1997) that apparently interact in local neural networks to process chemosensory information from the substrate (Gaffin and Brownell, 1997b). The peripheral organization of this sense organ is unique among arthropod chemosensory systems (Brownell, 1998) and appears to share structural similarities with the compound eye of insects (Strausfeld and Blest, 1970; Strausfeld 1970; Armett-Kibel et al., 1977) and the horseshoe crabs *Limulus polyphemus* (Fahrenbach, 1975; Fahrenbach, 1985).

The axonal appositions described in Golgi preparations may correspond to different types of peripheral synapses observed in the pectinal teeth (Foelix and Muller-Vorholt, 1983). At the ultrastructural level, axo-somatic and axo-axonic synapses have been described in the pectine (Foelix and Muller-Vorholt, 1983),

indicating that axonal swellings exhibiting this morphology may be peripheral synapses. Both excitatory and inhibitory interactions have been recorded in individual peg sensilla (Gaffin and Brownell, 1997b) but the synaptic organization of these sensory afferents and the functional significance of peripheral processing in the pectinal teeth is currently unknown and deserves further study.

Peripheral and Central Organization of Mechanosensory Afferents

Hair-like sensilla on the marginal and medial lamellae of the pectinal spine were classified by setae length into large, medium and small categories. Dendrites innervating large and medium hairs terminated at the base of the sensillar socket, resembling mechanoreceptors (Foelix and Chu-Wang, 1973a) and originated from a set of large (7-9 μm in diameter) bipolar neurons. Dendrites innervating small curved hairs were found inside the shaft of the sensillum, resembling chemoreceptors (Foelix and Chu-Wang, 1973b) and originated from clusters of smaller (4 μm in diameter) bipolar neurons.

The paired caudal neuropile serving the pectines (pectinal neuropile) contained two distinct regions: a basal disk and a terminal cap. The terminal cap was comprised of a fibrous cortex and a medullar region of numerous glomeruli. Sensory afferents from ablated patches of tactile hairs on the pectinal spine projected ipsilaterally in distinct tracts through the basal disk and along the cortex of the terminal cap. In contrast, sensory afferents from the pectinal teeth projected

ipsilaterally through the basal disk and terminated in both the cortex and medullar region.

The terminal cap of the pectinal neuropile appears divided into two regions (cortex and medulla) that process separate sensory modalities. Mechanosensory afferents from the pectinal spine were only found in the cortex. In contrast, mixed sensory afferents (chemosensory and mechanosensory) from the pectinal teeth projected into both regions. This differential pattern of innervation suggests that the cortex receives mechanosensory input and the medullar region receives chemosensory input. The internal architecture of the pectinal neuropile supports this hypothesis. The medullar region contained numerous glomeruli, a typical feature of chemosensory processing areas (Shephard, 1994; Hildebrand and Shephard, 1997).

Mechanosensory afferents from the pectinal spine may be represented somatotopically in the basal disk and cortex of the terminal cap. Sensory afferents from ablated mechanosensory hairs on the pectinal spine projected in distinct tracts through the pectinal neuropile in conserved positions. The number of ablated patches on the pectinal spine was consistent with the number labeled fiber tracts, suggesting that tactile afferents from the pectinal spine may be aligned in somatotopic register in the pectinal neuropile.

Sensory systems that organize chemical information in a spatial context are rarely found in nature. The pectines of scorpions are one example. The peg

sensilla on the pectinal teeth respond to mechanical and chemical stimuli (Hoffman 1964; Gaffin and Brownell, 1990; 1997a) and both types of afferents project topographically to the pectinal neuropile (Brownell 1989; 1998). The findings from the present study suggest that the pectinal neuropile may be divided into two regions that process separate sensory modalities. This pattern of neural organization suggests that two independent neural 'maps' are within the pectinal neuropile: one for substrate chemistry (medulla) and another for surface texture (cortex).

Evidence of Mate Trailing in Scorpions

Male *H. arizonensis* preferred the arm of the Y-maze that had been exposed to a reproductive female and did not show a significant preference for Y-maze arms exposed to other male conspecifics. When the substrate was repeatedly exposed to reproductive females, three behaviors were 'released' in male scorpions that resembled pre-courtship behaviors: juddering, tail-wagging and lunging. Tail wagging and lunging were only observed when the substrate was exposed to female conspecifics. Juddering was observed at a very low frequency (only 1 out of 25 trials) when the substrate was exposed to male conspecifics. Behavioral responses were not observed in negative controls (substrate not exposed to scorpions).

During the mating season, male desert scorpions are often found *wandering* dune environments, presumably in search of female conspecifics (Polis and Farley, 1980). Finding a receptive female is a potentially difficult and hazardous task

(Polis and Farley, 1979a; Polis and Sissom, 1990), suggesting that *wandering* by the male may be a form of guided mate-searching. The results from the Y-maze experiments support this hypothesis and demonstrate that giant hairy desert scorpions are capable of following the trails of female conspecifics. Male scorpions did not prefer maze arms that male scorpions had walked down, suggesting that mate trailing in scorpions may be mediated by a sex-specific signal that is chemical in origin.

Chemical cues appear to initiate courtship in *H. arizonensis*. Substrates exposed to female scorpions released pre-courtship behaviors in male conspecifics. In contrast, pre-courtship behaviors were not released in negative controls (substrates that were not exposed to scorpions). Juddering was released when the substrate was exposed to male conspecifics, but occurred in only 1 of 25 trials. Juddering is not limited to courtship in *H. arizonensis* (Tallarovic, 2000) and may be a form of intraspecific communication. Lunging has not been previously described and resembled mate-seizing behavior (Tallarovic, 2000). This behavior was only evoked when the male made contact with a wall or corner previously touched by the female. The results from this study suggest that female scorpions may deposit courtship pheromones on the substrate.

We have also developed a new model to study chemical communication in scorpions. Male *H. arizonensis* can discriminate the trail of a female conspecific in the Y-maze test (response rate > 60%), and produce pre-courtship behaviors (response rate > 60%) when exposed to sand that has been occupied by a

reproductive female. The Y-maze test, or a behavioral assay using the pre-courtship behaviors described, can now be used in a response-guided strategy (Albone, 1984; Mason, 1993) to isolate scorpion sex pheromones. The sensory structures involved in mate-trailing and sex pheromone detection could also be determined using the Y-maze test and reversible sensory blocking methods.

Bibliography

Abelson PH. 1985. Use of and research on pheromones. *Science* 229:1342.

Abushama FT. 1964. The behavior and sensory physiology of the scorpion *Leiurus quinquestriatus*. *Anim Behav* 12:1140-153.

Albone ES. 1984. Mammalian semiochemistry. Chichester: John Wiley and Sons.

Alexander AJ. 1957. The courtship and mating of the scorpion *Opisthophthalmus latimanus*. *Proc Zool Soc Lond* 128:529-44.

Alexander AJ. 1959. Courtship and mating in the buthid scorpions. *Proc Zool Soc Lond* 133:145-169.

Amakawa T, Kawata K, Ozaki M. 1992. Nucleotide receptor site on the labellar sugar receptor cell of the blowfly *Phormia regina*. *J Insect Physiol* 38:365-371.

Anderson JC, Laughlin SB. 2000. Photoreceptor performance and the co-ordination of achromatic and chromatic inputs in the fly visual system. *Vision Res.* 40:13-31.

Anton S, Barth FG. 1993. Central nervous projection patterns of trichobothria and other cuticular sensilla in the wandering spider *Cupiennius salei* (Arachnida, Araneae). *Zoomorphology* 217:129-136.

Anton S, Hannson BS. 1994. Central processing of sex pheromone, host odour, and oviposition deterrent information by interneurons in the antennal lobe of female *Spodoptera litoralis* (Lepidoptera: Noctuidae). *J Comp Neurol* 350:199-214.

Armett-Kibel C, Meinertzhagen IA, Dowling JE.1977. Cellular and synaptic organization in the lamina of the dragon-fly *Sympetrum rubicundulum*. *Proc R Soc Lond B* 196:385-413.

Atema J 1971. Structures and functions of the sense of taste in the catfish, *Ictalurus natalis*. *Brain Behav Evol* 4:273-294.

Atema J. 1985. Chemoreception in the sea: adaptations of chemoreceptors and behavior to aquatic stimulus conditions. *Soc Exp Biol Symp* 39: 387-423.

Abushama FT. 1964. The behavior and sensory physiology of the scorpion *Leiurus quinquestriatus*. Anim Behav 12:1140-153.

Babu KS. 1965. Anatomy of the central nervous system of arachnids. *Zool Jahrb Anat* 82:1-154.

Babu KS. 1985. Patterns of arrangement and connectivity in the central nervous system of arachnids. In: Barth FG, editor. *Neurobiology of Arachnids*. New York: Springer-Verlag. pp 3-19.

Babu KS, Barth FG. 1989. Central nervous projections of mechanoreceptors in the spider *Cupiennius salei*. *Cell Tissue Res* 258:69-82.

Bacon JP, Murphey RK. 1984. Receptive fields of cricket giant interneurons are related to their dendritic structures. *J Physiol Lond* 352:601-623.

Barbach JE, Case J. 1965. Sensory capabilities of the modified fins of the squirrel hake (*Urophycus chus*) and sea robins (*Prionotus carolinus* and *P. evolans*). *Copeia* 2:194-206.

Barre N, Naves M, Aprelon R, Fargetton M, L'Hostis M. 1998. Attractivity of cattle infested by *Amblyomma variegatum* (Acari: Ixodidae) for conspecific adult ticks from the field in Guadeloupe. *Exp Appl Acarol* 22: 297-308.

Barth FG. 1985. Neuroethology of the spider vibration sense. In: Barth FG, editor. *Neurobiology of Arachnids*. New York: Springer-Verlag. pp 203-229.

Barth FG, and Schmitt A. 1991. Species recognition and species isolation in wandering spiders (*Cupiennius* sp : Ctenidae). *Behav Ecol Sociobiol* 29:333-339.

Barth FG, Wastl U, Humphrey JAC, Devarakonda R. 1993. Dynamics of arthropod filiform hairs. II. Mechanical properties of spider trichobothria (*Cupiennius salei* KEYS.). *Phil Trans R Soc Lond B* 340:445-461.

Barth FG, Humphrey JAC, Wastl U, Halbritter J, Brittinger W. 1995 Dynamics of arthropod filiform hairs. III. Flow patterns related to air movement detection in a spider (*Cupiennius salei* Keys.). *Phil Trans R Soc Lond B* 347:397-412.

Barth FG, Holler A.1999. Dynamics of arthropod filiform hairs: the response of arthropod trichobothria to natural stimuli. *Phil Trans R Soc Lond B* 354:183-192.

Barth FG. 2000. How to catch the wind: spider hairs specialized for sensing the movement of air. *Naturwissenschaften* 87:51-58.

Bausenwein B, Fischbach KF. 1992. Separation of functional pathways in the fly's medulla: Combination of 2-deoxyglucose studies with anatomical fine analysis. In: Singh RN, editor. *Nervous systems: principles of design and function.* New Delhi: Wiley-Eastern. pp 223–239.

Bell WJ, Kipp LR, Collins RD. 1995. The role of chemo-orientation in search behavior. In: Carde RT, Bell WJ, editors. *Chemical Ecology of Insects 2*, New York: Chapman and Hall. pp 105-153.

Berger RS. 1972. 2,6-Dichlorophenol, sex pheromone of the lone star tick. *Science* 177:704-705.

Blaney WM, Chapman RF. 1969. The anatomy and histology of the maxillary palp of the locust, *Schistocerca gregaria* (Orthoptera, Acrididae). *J Zool* 157:509-535.

Boeckh J, Tolbert LP. 1993. Synaptic organization and development of the antennal lobe in insects. *Microsc Res Tech.* 24:260-80.

Bossert WH, Wilson EO. 1963. The analysis of olfactory communication among animals. *J Theor Biol* 4:443-469.

Braitenberg V 1967. Patterns of projection in the visual system of the fly. I. Retina-lamina projections. *Exp Brain Res* 3:271-298.

Breer H., Raming K, and Krieger J. 1994. Signal recognition and transduction in olfactory neurons. *Biochem Biophys Acta* 1224: 277-287.

Brownell PH, Farley RD. 1974. The organization of the malleolar sensory system in the solpugid, *Chanbria* sp. *Tissue Cell.* 6:471-585.

Brownell PH, Farley RD. 1979a. Detection of vibrations in sand by tarsal sense organs of the nocturnal scorpion *Paruroctonus mesaensis. J Comp Physiol (A)* 131:31-38.

Brownell PH, Farley RD. 1979b. Prey-localizing behavior of the nocturnal desert scorpion *Paruroctonus mesaensis*: orientation to substrate vibrations. *Anim Behav* 27:185-193.

Brownell PH. 1988. Properties and functions of the pectine chemosensory system of scorpions. *Chem Senses* 10:557.

Brownell PH. 1989. Neuronal organization and function of the pectinal sensory system in scorpions. *Soc Neurosci Abstr* 15:1289.

Brownell PH. 1998. Glomerular cytoarchitectures in chemosensory systems of arachnids. *Ann New York Acad Sci* 18:502-507

Brownell PH. 2000. Sensory ecology and orientational behaviors. In: Brownell PH, Polis G, editors. *Scorpion Biology and Research*. New York: Oxford Univ Press. pp 159-183.

Brusca RC, Brusca GJ. 1990. *Invertebrates*. Sunderland: Sinauer Associates Inc.

Buck L, Axel R 1991. A novel multigene family may encode odorant receptors: a molecular basis for odor recognition. *Cell* 65:175-167.

Burrows M, Siegler MVS. 1982. Spiking local interneurones mediate local reflexes. *Science* 217: 650-652.

Burrows M, Siegler MVS. 1984. The diversity and receptive fields of spiking local interneurones in the locust metathoracic ganglion. *J Comp Physiol (A)* 224: 483-508.

Burrows M, Newland PL. 1993. Correlation between the receptive fields of locust interneurons, their dendritic morphology, and the central projections of mechanosensory neurons. *J Comp Neurol* 329: 412-426.

Burrows M. 1996. The neurobiology of an insect brain. New York: Oxford University Press.

Buschbeck EK, Strausfeld NJ. 1996. Visual motion detection circuits in flies: small-field retinotopic elements responding to motion are evolutionarily conserved across taxa. *J Neurosci* 16:4563–4578.

Buschbeck EK, Strausfeld NJ. 1997. The relevance of neural architecture to visual performance: phylogenetic conservation and variation in dipteran visual systems. *J Comp Neurol* 383:282–304.

Cajal SR, Sanchez D. 1915. Contribucion al conocimiento de los insectos. *Trabajos del laboratorio de investigaciones biologicasde la universidad de Madrid.* 13:1–168.

Campos-Ortega JA, Strausfeld NJ. 1973. Synaptic connections of intrinsic cells and basket arborizations in the external plexiform layer of the fly's eye. *Brain Res* 59:119–136.

Carde RT. 1984. Chemorientation in flying insects. In Bell WJ, Carde RT, editors. *Chemical ecology of insects*. New York: Chapman and Hall. pp 111-121.

Carde RT, TC Baker. 1984. Sexual communication with pheromones. In: Bell W, Carde RT, editors. *Chemical Ecology of Insects*. New York: Chapman and Hall. pp 355-376.

Carthy JD. 1966. Fine structure and function of the sensory pegs on the scorpion pectine. *Experientia* 22:89-91.

Carthy JD. 1968. The pectines of scorpions. Symp Zool Soc Lond 23:251-261.

Caveney S. 1998. Compound eyes. In: Harrison FW, Locke M, editors. *Microscopic anatomy of invertebrates, V11B: Insecta*. New York: Wiley-Liss. pp 423-445.

Chapman RF. 1982. Chemoreception: the significance of sensillum numbers. *Adv Insect Physiol* 16: 247-356.

Chess A, Simon I, Cedar H, Axel R 1994. Allelic inactivation regulates olfactory receptor gene expression. *Cell* 78:823-834.

Chiba A, Kamper G, Murphey RK. 1992. Response properties of interneurons of the cricket cercal system are conserved in spite of changes in peripheral receptors during maturation. *J Exp Biol* 164:1-22.

Christensen TA, Hildebrand JG. 1987. Functions, organization, and physiology of the olfactory pathways in the lepidoteran brain. In: Gupta AP, editor. *The Arthropod Brain: its evolution, development, structure and functions*. New York: John Wiley and Sons. pp 457-483.

Christensen TA, Hildebrand JG. 1987. Male-specific, sex pheromone-selective projection neurons in the antennal lobes of the Moth, *Manduca sexta*. *J Comp Physiol (A)* 160: 553-569.

Christensen TA, Hildebrand JG. 1988. Frequency coding by central olfactory neurons in the sphinx moth *Manduca sexta*. *Chem Senses* 13(1): 123-130.

Christensen TA, Mustaparta H, Hildebrand JG. 1989. Discrimination of sex pheromone blends in the olfactory system of the moth. *Chem Senses* 14: 463-477.

Christensen TA, Hildebrand JG, Tumlinson JH, Doolittle RE. 1989. Sex pheromone blend of *Manduca sexta*: responses of central olfactory interneurons to antennal stimulation in male moths. *Arch Insect Biochem Physiol* 10: 281-291.

Christensen TA, Waldrop B, Harrow ID, Hildebrand JG. 1993. Local interneurons and information processing in the olfactory glomeruli of the moth, *Manduca sexta*. *J Comp Physiol (A)* 173:433-441.

Christensen TA, Harrow ID, Cuzzocrea C, Randolph PW, Hildebrand JG. 1995. Distinct projections of two populations of olfactory receptor axons in the antennal lobe of the sphinx moth *Manduca sexta*. *Chem Senses* 20: 313-323.

Cinelli AR, Kauer JS. 1994. Voltage-sensitive dyes and functional activity in the olfactory pathway. *Ann Rev Neurosci* 15:321-351.

Cloudsey-Thompson JL. 1955. On the function of the pectines of scorpions. *Ann Mag Nat Hist* 12 8:556-60.

Clyne PJ, Warr CG, Carlson JR. 2000. Candidate taste receptors in *Drosophila*. *Science*. 287:1830-1834.

Daddow LYM. 1986. An abbreviated method of the double lead stain technique. *J Submircosc Cytol Pathol* 18: 221-224.

Derby CD, Atema J. 1988. Chemoreceptor cells in aquatic invertebrates: peripheral mechanisms of chemical signal processing in decapod crustacea. In: Atema J, Popper AN, Fay RR, Tavolga WN, editors. *Sensory biology of aquatic animals*. New York: Springer-Verlag. pp 365-385.

Dethier VG. 1955. The physiology and histology of the contact chemoreceptors of the blowfly. *Q Rev Biol* 33:348–371.

Dethier VG. 1976. The Hungry Fly. Cambridge: Harvard Univ Press.

Diehl PA, Guerin PM, Vlimant M, Steullet P. 1991. Biosynthesis, production site, and emission rates of aggregation-attachment pheromone in males of two *Amblyomma ticks*. *J Chem Ecol* 17: 833-847.

Douglass JK, Strausfeld NJ. 1995. Visual motion detection circuits in flies: peripheral motion computation by identified small-field retino-topic neurons. *J Neurosci* 15:5596–5611.

Douglass JK, Strausfeld NJ. 1996. Visual motion-detection circuits in flies: parallel direction and nondirection-sensitive pathways between the medulla and lobula plate. *J Neurosci* 16:4551–4562.

Douglass JK, Strausfeld NJ. 1998. Functionally and anatomically segregated visual pathways in the lobula complex of a calliphorid fly. *J Comp Neurol* 396:84-104.

Dryer FC, Brockman JH. 1996. Sensory processes, orientation, and communication: biology of the Umwelt. In: Houck LD, Drickamer LC, editors. *Foundations of animal behavior: classic papers with commentaries*. Chicago: Univ Chicago Press. pp. 529-538.

Dubs A, Laughlin SB, Srinivasan MV. 1981. Single photon signals in fly photoreceptors and first order interneurons at behavioral threshold. *J Physiol Lond* 317:317–334.

Dunlop JA, Webster M. 1999. Fossil evidence, terrestrialization and arachnid phylogeny. *J Arachnol* 27:86-93.

Dusenberry DB. 1993. *Sensory ecology: how organisms acquire and respond to information*. New York: WH Freeman and Company.

Eckert H, Bishop LG. 1978. Anatomical and physiological properties of the vertical cells in the third optic ganglion of *Phaenicia sericata* (Diptera, Calliphoridae). *J Comp Physiol* 126:57–86.

Eckweiler W, Seyfarth EA. 1988. Tactile hairs and the adjustment of body height in wandering spiders: behavior, leg reflexes, and afferent leg projections in the leg ganglia. *J Comp Physiol (A)* 162:611-621.

Edgecomb RS, Murdock LL. 1992. Central projections of axons from taste hairs on the labellum and tarsi of the blowfly, *Phormia regina* Meigen. *J Comp Neurol* 315: 431-444.

Edwards JS, Palka J. 1974. The cerci and abdominal giant fibers of the house cricket *Acheta domesticus*. I. Anatomy and physiology of normal adults. *Proc R Soc Lond B* 185:83-103.

Elkington JS, Carde RT. 1984. Odor dispersion. In: Bell WJ, Carde RT, editors. *Chemical ecology of insects*. New York: Chapman and Hall. pp 73-88.

Ewing AW. 1989. Arthropod bioacoustics: neurobiology and behavior. New York: Cornell Univ Press.

Fabian-Fine R, Hoger U, Seyfarth EA, Meinertzhagen IA. 1999a. Peripheral synapses at identified mechanosensory neurons in spiders: three-dimensional reconstruction and GABA immunocytochemistry. *J Neurosci* 19:298-310.

Fabian-Fine R, Volknandt W, Seyfarth EA. 1999b. Peripheral synapses at identifiable mechanosensory neurons in the spider *Cupiennius salei*: synapsin-like immunoreactivity. *Cell Tissue Res* 295:9-13.

Fabre JH. 1923. The life of the scorpion. Tr. A. Teixeira de Mattos. New York: Dodd Mead.

Fahrenbach WH. 1975. The visual system of the horseshoe crab, *Limulus polyphemus*. *Int Rev Cytol* 41:285-349.

Fahrenbach WH. 1985. Anatomical circuitry of lateral inhibition in the eye of the horseshoe crab, *Limulus polyphemus*. *Proc R Soc Lond B* 225:219-249.

Fahrenbach WH. 1999. Merostomata. In: Harrison FW, Foelix RF, editors. *Microscopic anatomy of invertebrates. V8A: Chelicerate Arthropoda*. New York: Wiley-Liss. pp 1-115.

Falk R, Bleiser-Avivi N, Atidia J. 1976. Labellar taste organs of *Drosophila* melanogaster. *J Morphol* 150:327–342.

Farley RF. 1999. Scorpiones. In: Harrison FW, Foelix RF, editors. *Microscopic anatomy of invertebrates. V8A: Chelicerate Arthropoda*. New York: Wiley-Liss. pp 117-222.

Finger TE. 1982. Somatotopy in the representation of the pectoral fin and free fin rays in the spinal cord of the sea robin, *Prionotus carolinus*. *Biol Bull* 163:154-161.

Finger TE. 1997. Evolution of taste and solitary chemoreceptor cell systems. *Brain Behav Evol* 50:234-243.

Finger TE. 2000. Ascending spinal systems in the fish, *Prionotus carolinus*. *J Comp Neurol* 422:106-122.

Firestein S, Werblin F. 1989. Odor-induced membrane currents in vertebrate olfactory receptor neurons. *Science* 244:79-82.

Foelix RF, Axtell RC. 1972. Ultrastructure of the Haller's Organ in the tick *Amblyomma americanum* (L.). *Tissue Cell* 4:130-135.

Foelix RF, Chu-Wang I. 1973a. The morphology of spider sensilla: I. Mechanoreceptors. *Tissue Cell* 5:451-460.

Foelix RF, Chu-Wang I. 1973b. The morphology of spider sensilla: II. Chemoreceptors. *Tissue Cell* 5:461-478.

Foelix RF. 1975. Occurrence of synapses in peripheral nerves of arachnids. *Nature* 254:146-148.

Foelix RF, Choms A. 1979. Fine structure of a spider joint receptor and associated synapses. *Eur J Cell Biol* 19:149-159.

Foelix RF, Troyer D. 1980. Giant neurons and associated synapses in the peripheral nervous system of whip spiders. *J Neurocytol* 9:517-535.

Foelix RF, Muller-Vorholt G. 1983. The fine structure of scorpion sensory organs: II. Pectine sensilla. *Bull Brit Arachnol Soc* 6:68-74.

Foelix RF, Schabranath J. 1983. The fine structure of scorpion sensory organs. I. Tarsal sensilla. *Bull Brit Arachnol Soc* 6:53-67.

Foelix RF. 1985a. Mechano- and chemoreceptive sensilla. In: Barth FG, editor. *Neurobiology of Arachnids*. New York: Springer-Verlag. pp 118-137.

Foelix RF. 1985b. Sensory Nerves and Peripheral Synapses. In: Barth FG, editor. *Neurobiology of Arachnids*. New York: Springer-Verlag. pp 189-199.

Foelix RF. 1996. Biology of Spiders. New York: Oxford Univ Press.

Forel A. 1929. The social world of ants. New York: Boni press

Friedel T, Barth FG. 1997. Wind-sensitive interneurones in the spider CNS (*Cupiennius salei*): directional information processing of sensory inputs from trichobothria on the walking legs. *J Comp Physiol (A)* 180:223-233.

Gaffin DD, Brownell PH. 1990. Electrophysiological studies of the pectinal chemosensory system of scorpions. *Chem Senses* 15:579.

Gaffin DD, Brownell PH, Godde J. 1991. Electrophysiological evidence for synaptic interaction in single peg sensilla of scorpion pectines. *Chem Senses* 16:525.

Gaffin DD, Wennstrom KL, Brownell PH. 1992. Water detection in the desert sand scorpion *Paruroctonus mesaensis* (Scorpionida: Vaejovidae). *J Comp Physiol (A)* 170:623-629.

Gaffin DD, Brownell PH. 1997a. Response properties of chemosensory peg sensilla on the pectines of scorpiones. *J Comp Physiol (A)* 181:291-300.

Gaffin DD, Brownell PH. 1997b. Electrophysiological evidence of synaptic interactions within chemosensory sensilla of scorpion pectines. *J Comp Physiol (A)* 181:301-307.

Gaffin DD, Brownell PH. 2000. Chemosensory behavior and physiology. In: Brownell PH, Polis G, editors. *Scorpion Biology and Research*. New York: Oxford Univ Press. pp 223-245.

Gaskell WH. 1902. The origin of vertebrates, deduced from the study of ammocoetes, Part X. *J Anat Lond* 36:164-208.

Gesteland RC, Lettvin JY, Pitts WH. 1965. Chemical transmission in the nose of the frog. *J Physiol Lond* 181:525-559.

Ghysen A. 1980. The projection of sensory neurons in the central nervous system of *Drosophila*: choice of the appropriate pathway. *Dev Biol* 78:521-541.

Gilbert C, Strausfeld NJ. 1992. Small-field neurons associated with oculomotor and optomotor control in muscoid flies: cellular organization in the lobula plate. *J Comp Neurol* 316:56–71.

Gillary HL. 1966. Stimulation of the salt receptor of the blowfly. *J Gen Physiol* 50:359–368.

Giurfa M, Menzel R. 1997. Insect Visual perception: complex abilities of simple nervous systems. *Curr Opin Neurobiol* 7:505-513.

Gnatzy W, Tautz J. 1980. Ultrastructure and mechanical properties of an insect mechanoreceptor: stimulus-transmitting structures and sensory apparatus of the cercal filiform hairs of *Gryllus*. *Cell Tissue Res* 213:441-463.

Gnatzy W, Kamper G. 1990. Digger wasp against cricket. II. An airborne signal produced by a running predator. *J Comp Physiol (A)*. 167:551-556.

Goldrich NR. 1973. Behavioral responses of *Phormia regina* (Meigen) to labellar stimulation with amino acids. *J Gen Physiol* 61:74–88.

Gorb SN, Anton S, Barth FG. 1993. Central projections of cheliceral mechanoreceptors in the spider *Cupiennius salei* (Arachnida, Araneae). *J Morpholology* 217:129-136.

Gorner P. 1965. A proposed transducing mechanism for a multiply-innervated mechanoreceptor (trichobothrium) in spiders. *Cold Spr Harb Symp Quant Biol* 30:69-73.

Gray JR, Weeks JC. 1999. Anatomical correlates of a steroid induced change in synaptic strength during development. *Soc Neurosci Abstr* 25(1-2):124.

Griffin AJ, Fahrenbach WH. 1977. Gill receptor arrays in the horseshoe crab (*Limulus polyphemus*). *Tissue Cell* 9:745-750.

Gronenburg W. 1989. Anatomical and physiological observations on the organization of mechanoreceptors and local interneurons in the central nervous system of the wandering spider *Cupiennius salei*. *Cell Tissue Res* 258:163-175.

Gronenburg W. 1990. The organization of plurisegmental mechanosensitive interneurons in the central nervous system of the wandering spider *Cupiennius salei*. *Cell Tissue Res* 260:49-61.

Hangartner W. 1967. Spezifitat und inaktivierung des spurpheromons von *Lassius fuliginosus* Latr. und oreintierung der arbeiterinnen in duftfeld. *Zeit Vergl Physiol* 62:111-120.

Hannson BS, Christensen TA, Hildebrand JG. 1991. Functionally distinct subdivisions of the macroglomerular complex in the antennal lobe of the male sphinx moth *Manduca sexta*. *J Comp Neurol* 312:264-278.

Hannson BS, Ljungberg H, Hallberg E, Lofstedt C. 1992. Functional specialization of olfactory glomeruli in a moth. *Science* 256:1313-1315.

Hannson BS, Anton S. 2000. Function and morphology of the antennal lobe: new developments. *Annu Rev Entomol* 45:205-231.

Hanstrom B. 1923. Further notes on the central nervous system of arachnids: scorpions, phalangids and trap door spiders. *J Comp Neurol* 35:249-272.

Hardie RC, Kirschfeld K. 1983. Ultraviolet sensitivity of fly photoreceptors R7 and R8: evidence for a sensitizing function. *Biophys Struc Mech* 9: 171–180.

Hardie RC. 1985. Functional organization of the fly retina. *Prog Sen Physiol* 5:1–79.

Hardie RC. 1986. The photoreceptor array of the Dipteran retina. *Trends Neurosci* 9:419–423.

Harris DJ, Mill PJ. 1977a. Observations on the leg receptors of *Cniflio* (Araneidae: Dictynidae). I. External mechanoreceptors. *J Comp Physiol* 119:37-54.

Harris DJ, Mill PJ. 1979b. Observations on the leg receptors of *Cniflio* (Araneida, Dictynidae). II. Chemoreceptors. *J Comp Physiol* 119:37-54.

Hartline HK, Ratliff F. 1974. Inhibitory interactions in the retina of Limulus. In: F Ratliff F, editor. *Studies on excitation and inhibition in the retina*. New York: Rockefeller Univ Press. pp 381-447.

Hausen K. 1984. The lobula complex of the fly: structure, function, and significance in visual behavior. In: Ali MA, editor. *Photoreception and vision in invertebrates*. New York: Plenum Press. pp 523–599.

Hausen K. 1993. Decoding of retinal image flow in insects. In: Miles FA, Wallman J, editors. *Visual motion and its role in the stabilization of gaze*. New York: Elsevier Science Publishers. pp 203–235.

Hayama T, Caprio J. 1989. Lobule structure and somatotopic organization of the medullary facial lobe in the channel catfish *Ictalurus punctatus*. *J Comp Neurol* 285:9-17.

Hayes WF. 1971. Fine structure of the Chemoreceptor sensillum in *Limulus*. *J Morphol* 133:205-240.

Hayes WF, Barber SB. 1982. Peripheral synapses in *Limulus* chemoreceptors. *J Comp Biochem Physiol* 72A:287-293.

Hegdekar BM, Dondale CD. 1969. A contact sex pheromone and response parameters in Lycosid spiders. *Can J Zool* 47:1-4.

Heiligenberg W. 1991. The neural basis of behavior: a neuroethological view. *Annu Rev Neurosci* 14:247-267.

Herrick CJ. 1907. The tactile centers in the spinal cord and brain of the sea robin, *Prionotus carolinus*. *J Comp Neurol Psychol* 17:307-327.

Hertel H. 1980. Chromatic properties of identified interneurons in the optic lobes of the bee. *J Comp Physiol (A)*. 137:215-232.

Hertel H, Maronde U. 1987. Processing of visual information in the honey bee brain. In: Menzel R, Mercer A. editors. *Neurobiology and behavior of the honey bee*. New York: Spring-Verlag. pp 141-151.

Hess E, Vlimant M. 1982. The tarsal sensory system of *Amblyomma variegatum* Fabricius (Ixodidae, Metastriata). I. Wallpore and terminal pore sensilla. Rev *Suisse Zool* 89: 713-729.

Hildebrand JG. 1995. Analysis of chemical signals by nervous systems. *Proc Natl Acad Sci USA* 92:67-74.

Hildebrand JG, Shephard GM. 1997. Mechanisms of olfactory discrimination: converging evidence for common principles across phyla. *Annu Rev Neurosci* 20:595-631

Hjelle JT. 1990. Anatomy and morphology. In: Polis GA, editor. *The biology of scorpions*. Stanford: Stanford Univ Press. pp 9-63.

Hoffman C. 1964. Zur funktion der kammformigen organ von skorpionen. *Naturwissenschaften* 7:172.

Hoger U, Barth FG. 1995. Just in the nick of time: postembryonic development of tactile hairs and of tactile behavior in spiders. *Zoology* 99:49-57.

Holldobler B, Wilson EO. 1970. Recruitment trails in the harvester ant *Pogonomyrmex badius*. *Psyche* 77:385-399.

Holldobler B, Traniello JFA. 1980. The pygidial gland and chemical recruitment communication in *Pachycondyla laevigata*. *J Chem Ecol*. 6:883-893.

Holldobler B, Wilson EO. 1990. The ants. Cambridge: Belknap press.

Homberg U, Montague RA, Hildebrand JG. 1988. Anatomy of the antenno-cerebral pathways in the brain of the sphinx moth *Manduca sexta*. *Cell Tissue Res* 254:255-281.

Homberg U, Christensen TA, Hildebrand. 1989. Structure and function of the deuterocerebrum in insects. *Annu Rev Entomol* 34:477-501.

Horridge GA .1977. The compound eye of insects. *Sci Amer* 237(1):108-20.

Humphrey CD, Pittman FE. 1974. A simple methylene blue-azure II-basic fuschin stain for epoxy embedded sections. *Stain Technol* 42:9-14.

Igelmund P, Wendler G. 1991a. Morphology and physiology of peripheral giant interneurons in the forelegs (whips) of the whip spider *Heterophrynus elaphus* Pocock (Arachnida : Amblypygi). *J Comp Physiol (A)* 168:75-83.

Igelmund P, Wendler G. 1991b. The giant fiber system in the forelegs (whips) of the whip spider *Heterophrynus elaphus* Pocock (Arachnida : Amblypygi). *J Comp Physiol (A)* 168:63-73.

Imamura K, Mataga N, Mori K. 1992. Coding of odor molecules by mitral/tufted cells in rabbit olfactory bulb. I. Aliphatic compounds. *J Neurophysiol* 68:1986-2002.

Ishimoto H, Matsumoto A, Tanimura T. 2000. Molecular identification of a taste receptor gene for trehalose in *Drosophila*. *Science* 289:116-9.

Jackson PR. 1987. An analysis of alternative mating tactics of the jumping spider, *Phidippus johnsoni* (Araneae, Salticidae). *J Arachnol* 5:185-230.

Jacobs GA, Miller JP, Murphey RK. 1986. Cellular mechanisms underlying directional sensitivity of an identified sensory interneuron. *J Neurosci* 6:2298-2311.

Jacobs GA, Nevin R. 1991. Anatomical relationships between sensory afferent arborizations in the cricket cercal system. *Anat Rec* 231:563-572.

Jacobs GA, Theunissen FE. 1996. Functional organization of a neural map in the cricket cercal sensory system. *J Neurosci* 16:769-784.

Jacobs GA, Theunissen FE. 2000. Extraction of sensory parameters from a neural map by primary sensory interneurons. *J Neurosci* 20:2934-2943.

Jarvilehto M, Zettler F. 1973. Electrophysiological-histological studies on some functional properties of visual cells and second order neurons of an insect retina. *Z Zellforsch* 136:291-306.

Johnson SE, Murphy RK. 1985. The afferent projection of mesothoracic bristle hairs in the cricket, Acheta domesticus. *J Comp Physiol (A)* 156:369-379.

Kaissling KE. 1986. Chemo-electrical transduction in insect olfactory receptors. *Annu Rev Neurosci* 9: 121-145.

Kaissling KE, Hildebrand JG, and Tumlinson JH. 1989. Pheromone receptor cells in the male moth, *Manduca sexta. Arch Insect Biochem Physiol* 10: 273-279.

Kanwal JS, Hidaka I, Caprio J. 1987. Taste responses to amino acids from the facial nerve branches innervating oral and extra-oral taste buds in the channel catfish, *Ictalurus puntatus. Brain Res* 406:105-112.

Kanzaki R., Arbas EA, Hildebrand JG. 1991. Physiology and morphology of protocerebral olfactory neurons in the male moth *Manduca sexta. J Comp Physiol (A)* 168: 281-298.

Kennedy JS, Ludlow AR, Sanders CJ. 1980. Guidance system used in moth sex attraction. *Nature* 288: 475-477.

Kennedy JS. 1983. Zigzagging and casting as a programmed response to wind borne odour: a review. *Physiol Entomol* 8: 109-120.

Kent KS, Levine RB. 1988. Neural control of leg movements in a metamorphic insect: sensory and motor elements of the larval thoracic legs in *Manduca sexta. J Comp Neurol* 271:559-576.

King RC, Christensen TA, Hildebrand JG. 2000. Response characteristics of an identified, sexually dimorphic olfactory glomerulus. *J Neurosci* 20:2391-2399.

Kirschfeld. 1967. Die projection der optisehen Umwelt auf das raster der rhabdomere in komplexauge von *Musca. Exp Brain Res* 3:248-270.

Kjellesvig-Waering EN. 1986. A restudy of the fossil scorpionida of the world. *Paleontograph Americana* 55.

Klaggs BRE, Heimbeck G, Godenschwege TA, Hofbauer A, Pflugfelder GO, Reifegerste R, Reisch D, Schaupp M, Buchner S, Buchner E. 1996. Invertebrate synapsins: a single gene codes for several isoforms in *Drosophila. J Neurosci* 16:3154-3165.

Klein U. 1981. Sensilla of the cricket palp. Fine structure and spatial organization. *Cell Tissue Res* 219: 229-252.

Kraeplin K. 1907. Die sekundaren geschlechtscharaktere der scorpion, pediplapen und solifugen. *Mitteilungen aus dem Naturhistorichen Museum in Hamburg* 25:185-225.

Krapf D. 1986. Contact chemoreception of prey in hunting scorpions. (Arachnida:Scorpiones). *Zool Anz* 217:119-129.

Lancet D. 1986. Vertebrate olfactory reception. *Annu Rev Neurosci* 9:329-355.

Land MF. 1985. The eye: optics. In: Kerkut GA, Gilbert LI, editors. *Comprehensive insect physiology, biochemistry and pharmacology. V6 Nervous system: sensory.* New York: Pergamon press. pp 225-276.

Landolfa MA, Miller JP. 1995. Stimulus-response properties of cricket cercal filiform receptors. *J Comp Physiol (A)* 177:747-757.

Laughlin SB, Hardie RC. 1978. Common strategies for light adaptation in the peripheral visual systems of fly and dragonfly. *J Comp Physiol* (A). 128, 319–340.

Laughlin SB. 1981. Neural principles in the peripheral visual systems of invertebrates. In: Autrum H, editor. *Comparative physiology and evolution of vision in invertebrates. Handbook of sensory physiology VII/6B.* Berlin: Springer. pp 133–280.

Laughlin SB. 1983. The roles of parallel channels in early visual processing by the arthropod compound eye. In: Ali MA, editor. Photoreception and vision in invertebrates. New York: Plenum Press. pp 457-81.

Laughlin SB. 1989. Coding efficiency and design in visual processing. In: Stavenga DG, Hardie RC, editors. *Facets of Vision*. Berlin: Springer-Verlag. pp 213–234.

Laughlin SB. 1999. Dendritic integration makes sense of the world. *Curr Biol* 9:15-17.

Laurent G. 1999. A systems perspective on early olfactory coding. *Science* 286: 723-728.

Leveteua J, Macleod P. 1966. Olfactory discrimination in the rabbit olfactory glomerulus. *Science* 175:170-178.

Levine RB, Pak C, Linn D. 1985. The structure, function and metamorphic reorganization of somatotopically projecting sensory neurons in *Manduca sexta* larvae. *J Comp Physiol (A)* 157:1-13.

Lewis DB, Gower DM. 1980. Biology of Communication. New York: Wiley-Liss.

Linn CE, Bjostad B, Du JW, Roelofs WL. 1984. Redundancy in a chemical signal: behavioral responses of male *Trichoplusiani* to a 6-component sex pheromone blend. *J Chem Ecol* 10:1653-1658.

Linn CE, Cambell MG, Roelofs WL. 1986. Male moth sensitivity to multicomponent pheromones: critical role of female released blend in determining the functional role of components and active space of the pheromone. *J Chem Ecol* 12:659-668.

Lloyd JE. 1983. Bioluminescence and communication in insects. *Annu Rev Entomol* 28:131-160.

Maccary A. 1810. Memoire sur le scorpion qui se trouve sur la montagne de Cette. Paris: Gabon.

Machan L. 1968. Spectral sensitivity of scorpion eyes and the possible role of shielding pigment effect. *J Exp Biol* 49:95-105.

Malnic B, Hirono J, Sato T, Buck LB. 1999. Combinatorial receptor codes for odors. *Cell* 96:713-723

Marui T, Caprio J. 1982. Electrophysiological evidence for the topographical arrangement of taste and tactile neurons in the facial lobe of the channel catfish. *Brain Res* 231:185-190.

Mason RT. 1993. Chemical ecology of the red-sided garter snake, *Thamnophis sirtalis parietalis*. *Brain Behav Evol.* 41:261-268.

McCormick SJ, Polis GA. 1990. Predators Prey and Parasites. In: Polis GA, editor. *The biology of scorpions*. Stanford: Stanford Univ Press. pp 161-223.

McCutchan MC. 1969a. Responses of tarsal chemoreceptive hairs of the blowfly, *Phormia regina*. *J Insect Physiol* 15:2059–2068.

McCutchan MC. 1969b. Behavioral and electrophysiological responses of the blowfly, *Phormia regina* Meigen, to acids. *Z Vergl Physiol* 65:131–152.

McMahon C, Guerin PM. 2000. Responses of the tropical bont tick, *Amblyomma variegatum* (Fabricius), to its aggregation-attachment pheromone presented in an air stream on a servosphere. *J Comp Physiol (A)* 186: 95-103.

Melamed J, Trujillo-Cenoz O. 1969. The fine structure of the central cells in the ommatidia of Dipterans. *J Ultrastruct Res* 21:313-334.

Mellon D, Munger SD. 1990. Nontopographic projection of olfactory sensory neurons in the crayfish brain. *J Comp Neurol* 296:253-262.

Melville JM, Brownell PH. 1997. Laminar cytoarchitecture and topography of sensory neurons in the scorpion pectine. *Soc Neurosci Abstr* 23(1-2):768.

Melville JM, Tallarovic SK, Gundersen LE, and Brownell PH. 1999. Evidence of mate trailing in the giant hairy desert scorpion. *Anim Behav Soc Abstr* P68:53-54.

Mesce KA, Klukas KA, Brelje TC. 1993. Improvements for the anatomical characterization of insect neurons in whole mount: the use of cyanine-derived fluorophores and laser scanning confocal microscopy. *Cell Tissue Res* 271:381-397.

Michel WC, Kohabra J, Caprio J. 1993. Amino acid receptor sites in the facial taste system of the sea catfish, *Arius felis*. *J Comp Physiol (A)* 172:129-138.

Miller JP, Jacobs GA, Theunissen FE. 1991. Representation of sensory information in the cricket cercal sensory system. I. Response properties of the primary interneurons. *J Neurophysiol* 66:1680-1689.

Mitchell BK, Itagaki H. 1992. Interneurons of the subesophageal ganglion of *Sarcophaga bullata* responding to gustatory and mechanosensory stimuli. *J Comp Physiol (A)* 171:213-230.

Mitchell BK, Itagaki H, Rivet M. 1999. Peripheral and central structures involved in insect gustation. *Microsc Res Tech* 47:401-415.

Mombaerts P, Wang F, Dulac C, Vassar R, Chao SK, Nemes A. 1996. Visualizing an olfactory sensory map. *Cell* 87:675-86.

Mombaerts P. 1999. Molecular biology of odorant receptors in vertebrates. *Annu Rev Neurosci* 22:487-509.

Mori K, Mataga N, Imamura K. 1992. Differential specificities of single mitral cells in rabbit olfactory bulb for a homologous series of fatty acid odor molecules. *J Neurophysiol* 67:786-789.

Mori HN, Yoshihiro Y. 1999. The Olfactory Bulb: Coding and Processing of Odor Molecule Information. *Science* 22: 286: 711-715.

Morrill AD. 1895. The pectoral appendages of *Prionotus* and their innervation. *J Morpholol* 11:177-192.

Murlis J, Jones CD. 1981. Fine scale structure of odor plumes in relation to insect orientation to distant pheromone and other attractant sources. *Physiol Entomol* 6:71-86.

Murphey RK. 1981. The structure and development of a somatotopic map in crickets: the cercal afferent projection. *Dev Biol* 88:236-246.

Murphey RK, Possidente D, Pollack G, Merritt DJ. 1989. Modality specific axonal projections in the CNS of the flies *Phormia* and *Drosophila. J Comp Neurol* 290:185-200.

Nagayama T, Burrows M. 1990. The organization of receptive fields of an antero-medial group of spiking local interneurons in the locust metathoracic ganglion. *J Comp Physiol (A)* 166:471-476.

Nayak SV, Singh RN. 1985. Primary sensory projections from the labella to the brain of *Drosophila melanogaster* Meigen (Diptera: Drosophilidae). *Int J Insect Morphol Embryol* 14:115-129.

Nayak, SV, Singh RN. 1983. Sensilla on the tarsal segments and mouthparts of adult *Drosophila melanogaster* Meigen (Diptera: Drosophilidae). *Int J Insect Morphol Embryol. 12:273–291.*

Newland PL. 1991a. Physiological properties of afferents from tactile hairs on the hindleg of locusts. *J Exp Biol* 155:487-403.

Newland PL. 1991b. Morphology and somatotopic organization of the central projections of afferents from tactile hairs on the hind leg of the locust. *J Comp Neurol* 311: 1-16.

Newland PL, Burrows M. 1994. Processing of mechanosensory information from gustatory receptors on a hind leg of the locust. *J Comp Physiol (A)* 174: 399-410.

Newland PL. 1998. Avoidance reflexes mediated by contact chemoreceptors on the legs of locusts. *J Comp Physiol (A)* 183: 313-324.

Newland PL. 1999. Processing of gustatory information by spiking local interneurones in the locust. *J Neurophysiol* 82: 3149-3159.

Newland PL, Rogers SM, Gaaboub I, Matheson T. 2000. Parallel somatotopic maps of gustatory and mechanosensory neurons in the central nervous system of an insect. *J Comp Neurol* 425:82-96.

Norval RAI, Andrew HR, Yunker CE. 1989. Pheromone mediation of host-selection in bont ticks (*Amblyomma hebraeum* Koch). *Science* 243: 364-365.

Olberg RM, Miller JP. 1991. Behavioral measurements of directional resolution in the cricket cercal escape system. *Soc Neurosci Abstr* 17:639.

Ozaki M, Amakawa T, Ozaki K, Tokunaga F. 1993. Two types of sugar-binding protein in the labellum of the fly: putative taste receptor molecules for sweetness. *J Gen Physiol* 102:201–216.

Paydar S, Doan CA, Jacobs GA. 1999. Neural mapping of direction and frequency in the cricket cercal sensory system. *J Neurosci* 19:1771-1781.

Peterson BA, Weeks JC. 1988. Somatotopic mapping of sensory neurons innervating mechanosensory hairs on the larval prolegs of *Manduca sexta*. *J Comp Neurol* 275:128-144.

Pflüger HJ. 1980. The function of hair sensilla on the locust's leg: the role of tibial hairs. *J Exp Biol* 87: 263-175.

Pflüger HJ, Bräunig P, Hustert R. 1988. The organization of mechanosensory neuropiles in locust thoracic ganglia. *Philos Trans R Soc Lond B* 321: 1-26.

Polis GA, Farley RD. 1979. Behavior and ecology of mating in the cannibalistic scorpion *Paruroctonus mesaensis* (Stahnke) (Scorpionida:Vaejovidae). *J Arachnol* 7:33-46.

Polis GA. 1980. Seasonal patterns and age-specific variation in the surface activity of a population of desert scorpions in relation to environmental factors. *J Anim Ecol* 49:1-18.

Polis GA, McCormick SJ. 1987. Intraguild predation and competition among desert scorpions. *Ecology* 68:323-343.

Polis GA. 1990. Ecology. In: Polis GA, editor. *The biology of scorpions*. Stanford: Stanford Univ Press. pp 161-223.

Polis GA, Sissom WD. 1990. Life history. In: Polis GA, editor. *The biology of scorpions*. Stanford: Stanford Univ Press. pp 161-223.

Pollack GS, Balakrishnam R. 1997. Taste sensilla of flies: function, central neuronal projections and development. *Microsc Res Tech* 39:532-546.

Pollard SD, Macnab AM, Jackson RR. 1987. Communication with chemicals: pheromones and spiders. In: Nentwig W, editor. *Ecophysiology of spiders*. New York: Springer-Verlag. pp 133-141.

Prouvost O, Trabalon M, Papke M, Schulz S. 1999. Contact sex signals on web and cuticle of *Tegenaria atrica* (Araneae, Agelenidae). *Arch Insect Biochem Physiol* 40:194-202.

Reissland A, Gorner P. 1985. Trichobothria. In: Barth FG, editor. *Neurobiology of arachnids*. New York: Springer-Verlag. pp 138-161.

Roddey JC, Jacobs GA. 1996. Information theoretic analysis of dynamical encoding by filiform mechanoreceptors in the cricket cercal system. *J Neurophysiol* 75:1365-1376.

Rodrigues V, Siddiqi O. 1981. A gustatory mutant of *Drosophila* defective in pyranose receptors. *Mol Gen Genet* 181: 406-408.

Roeder KD. 1963. Nerve cells and insect behavior. Harvard Univ Press. Cambridge Mass.

Rogers SM, Newland PL. 2000. Local movements evoked by chemical stimulation of the hind leg in the locust, *Schistocerca gregaria*. *J Exp Biol* 203: 423-433.

Roland C. 1984. Chemical signals bound to the silk in spider communication (Arachnida, Araneae). *J Arachnol* 11:309-314.

Root TH. 1990. Neurobiology. In: Polis GA, editor. *The biology of scorpions*. Stanford: Stanford Univ Press. pp 341-409.

Rosin R, Shulov A. 1963. Studies on the scorpion *Nebo hierichonticus*. *Proc Zool Soc Lond* 140:547-575.

Rovner JS, Barth FG. 1981. Vibratory communication through living plants by a tropical wandering spider. *Science* 214:464-466.

Sanes JR, Hildebrand JG. 1976. Origin and morphogenesis of sensory neurons in an insect antennae. *Dev Biol* 51:300-319.

Scharrer E, Smith SW, Palay SL. 1947. Chemical senses and taste in the fishes *Prionotus* and *Trichogaster*. *J Comp Neurol* 86:183-198.

Schmidt M, Ache BW. 1996. Processing of antenullar input in the brain of the spiny lobster, *Panulirus argus*, II the olfactory pathway. *J Comp Physiol (A)* 178: 605-628.

Schneider D. 1992. 100 years of pheromone research. An essay on Lepidoptera. *Naturwissenschaften* 79:241-250.

Schnuch M, Seebauer H. 1998. Sugar cell responses to lactose and sucrose in labellar and taste hairs of *Musca domestica*. *J Comp Physiol (A)* 182:767-775.

Schoeni R, Hess E, Blum W, Ramstein K. 1984. The aggregation attachment pheromone of the tropical bont tick *Amblyomma variegatum* Fabricius (Acari: Ixodidae): isolation, identification and action of its components. *J Insect Physiol* 30:613-618.

Schroder O. 1908. Der sinnesorgane der skorpionskamme. *Z Wiss Zool* 9:436-444.

Schultz S, Toft S. 1993. Identification of a sex pheromone from a spider. *Science* 260:1635-1637.

Schultz S. 1997. The chemistry of spider toxins and spider silk. *Angew Chem Int Ed Engl* 36:314-326.

Shanbhag SR, Singh RN. 1992. Functional implications of the projections of neurons from individual labellar sensillum of *Drosophila melanogaster* as revealed by neuronal-marker horseradish peroxidase. *Cell Tissue Res.* 267:273–282.

Shaw SR. 1989. The retina-lamina pathways in insects, particularly diptera, viewed from an evolutionary perspective. In: Stavenga DG, Hardie RC, editors. *Facets of Vision*. Berlin: Springer-Verlag. pp 186–212.

Shephard GM, Firestein S. 1992. Making scents of olfactory transduction. *Curr Biol* 1:204-206.

Shepherd GM. 1994. Discrimination of olfactory signals by the olfactory receptor neuron. *Neuron* 13:771-790.

Shimada I, Shiraishi A, Kijima H, Morita H. 1974. Separation of two receptor sites in a single labellar sugar receptor of the flesh-fly by treatment with p-chloromecuribenzoate. *J Insect Physiol* 20:605–621.

Shimada I, Isono K. 1978. The specific receptor site for aliphatic carboxylate anion in the labellar sugar receptor of the fleshfly. *J Insect Physiol* 24:807–811.

Shimada I, Horiki H, Ohrui H, Meguro H. 1985. Taste response to 2,5-anhydro-D-hexitols; rigid stereospecificity of the furanose site in the sugar receptor of the flesh fly. *J Comp Physiol (A)* 157:477–482.

Shiraishi A, Kuwabara M. 1970. The effects of amino acids on the labellar hair chemosensory cells of the fly. *J Gen Physiol* 56:768–782.

Siddiqi O, Joshi S, Arora K, Rodrigues V. 1989. Genetic investigation of salt reception in *Drosophila melanogaster*. *Genome*, 31:646–651.

Siegler MVS, Burrows M. 1983. Spiking local interneurons as primary integrators of mechanosensory information in locust. *J Neurophysiol* 50: 1281-1295.

Siegler MVS, Burrows M. 1986. Receptive fields of motor neurons underlying local tactile reflexes in the locust. *J Neurosci* 6: 507-513.

Silver WL, Finger TE. 1984. Electrophysiological examination of a non-olfactory, non-gustatory chemosense in the sea robin, *Prionotus carolinus*. *J Comp Physiol (A)* 154:167-174.

Singh RN. 1997. Neurobiology of the gustatory systems of *Drosophila* and some terrestrial insects. *Micrsoc Res Tech* 39:547-563.

Sissom D. 1990. Systematics, Biogeography and Paleontology. In: Polis GA, editor. *The biology of scorpions*. Stanford: Stanford Univ Press. pp 161-223.

Slifer EH. 1970. The structure of arthropod chemoreceptors. *Annu Rev Entomol* 15:121-142.

Sonenshine DE. 1985. Pheromones and other semiochemicals of the Acari. *Annu Rev Entomol* 30:1-28.

Sonenshine DE. 1991. Biology of Ticks. New York: Oxford Univ Press.

Srinivasan MV, Zhang SW, Rolfe B. 1993. Is pattern vision in insects mediated by ''cortical'' processing? *Nature* 362:539–540.

Stahnke HL. 1972a. UV light, a useful field tool. *Bioscience* 22:604-607.

Stahnke HL. 1974. Revision and keys to higher categories of Vaejovidae. J *Arachnol* 1:107-141

Steullet P, Guerin PM. 1992a. Perception of breath components by the tropical bont tick, *Amblyomma variegatum* Fabricius (Ixodidae) I. CO_2 -excited and CO_2 -inhibited receptors. *J Comp Physiol (A)* 170: 665-676.

Steullet P, Guerin PM. 1992b. Perception of breath components by the tropical bont tick *Amblyomma variegatum* Fabricius (Ixodidae) II. Sulfide receptors. *J Comp Physiol (A)* 170: 677-685.

Steullet P, Guerin PM. 1994a. Identification of vertebrate volatiles stimulating olfactory receptors on tarsus I of the tick *Amblyomma variegatum* Fabricius (Ixodidae) II. Receptors outside the Haller's organ capsule. *J Comp Physiol (A)* 174: 39-47.

Steullet P, Guerin PM. 1994b. Identification of vertebrate volatiles stimulating olfactory receptors on tarsus I of the tick *Amblyomma variegatum* Fabricius (Ixodidae) I. Receptors within the Haller's organ capsule. *J Comp Physiol (A)* 174: 27-38.

Stocker RF, Schroderet M. 1981. Cobalt filing of sensory projections from internal and external mouthparts in *Drosophila. Cell Tissue Res* 216:513-523.

Stormer L. 1969. Oldest known terrestrial arachnids. *Science*. 164:1276-1277.

Strausfeld NJ. 1970. Golgi studies on insects. Part II. The optic lobes of Diptera. *Philosophic Trans R Soc Lond B* 258:135-223.

Strausfeld NJ, Blest AD. 1970. Golgi studies on insects. Part I. The optic lobes of Lepidoptera. *Philosophic Trans R Soc Lond B* 258:82-134.

Strausfeld NJ, Campos Ortega JA. 1973. L3, the 3rd 2nd-order neuron of the 1st visual ganglion in the "neural superposition" eye of *Musca domestica. Z Zellforsch.* 139:397-403.

Strausfeld NJ. 1976. Atlas of an insect brain. Berlin: Springer.

Strausfeld NJ, Campos Ortega JA. 1977. Vision in insects: pathways possibly underlying neural adaptation and lateral inhibition. *Science* 195:894-897.

Strausfeld NJ. 1980. The Golgi method: Its application to the insect nervous system and the phenomenon of stochastic impregnation. In: Strausfeld NJ, Miller TA, editors. *Neuroanatomical techniques for the insect nervous system.* New York: Springer-Verlag pp. 132-190.

Strausfeld NJ. 1989. Beneath the compound eye: neuroanatomical analysis and physiological correlates in the study of insect vision. In: Stavenga DC, Hardie RC, editors. *Facets of Vision.* Berlin: Springer-Verlag. pp 317–359.

Strausfeld NJ, Gilbert C. 1992. Small-field neurons associated with oculomotor and optomotor control in muscoid flies: functional organization. *J Comp Neurol* 316:72–86.

Strausfeld NJ, Lee JK. 1991. Neuronal basis for parallel visual processing in the fly. *Visual Neurosci* 7:13–33.

Strausfeld NJ, Hansen L, Li Y, Gomez RS, Ito K. 1998. Evolution, discovery, and interpretations of arthropod mushroom bodies. *Learn Mem* 5:11-37.

Suter RB, Renkes G. 1982. Linyphiid spider courtship: releaser and attractant functions of a contact sex pheromone. *Anim Behav* 30:714-718.

Suter RB, Hirscheimer AJ. 1986. Multiple web-borne pheromones in a spider *Frontinella pyramitella* (Araneae: Linyphiidae). *Anim Behav* 34:748-753.

Suter RB, Shane CM, Hirscheimer AJ. 1987. Communication by cuticular pheromones in a Linyphiid spider. *J Arachnol* 15:157-162.

Swoveland MC. 1978. External morphology of the scorpion pectines. Masters thesis, California State University, San Francisco.

Szlendak E, Oliver JH. 1992. Anatomy of synganglia, including their neurosecretory regions, in unfed, virgin female *Ixodes scapularis* Say (Acari: Ixodidae). *J Morphol* 213:349-364.

Tallarovic SK. 2000. Conspecific and mating behaviors of the giant hairy desert scorpion, Ph.D. Dissertation, Oregon State University, Corvallis.

Tanimura T, Shimada I. 1981. Multiple receptor proteins for sweet taste in *Drosophila* discriminated by papain treatment. *J Comp Physiol (A).* 141:265–269.

Taylor PW. 1998. Dragline-mediated mate searching in *Trite planiceps* (Araneae, Salticidae). *J Arachnol* 26:330-334.

Thevenieau Laurent. 1999. Rapport de stage pheromones de contact. Maitrise de biologie cellulaire et de physiologie, Universite de la Mediterranee, Aix Marseille II.

Tietjen WJ. 1977. Dragline-following by male lycosid spiders. *Psyche* 84:165-178.

Tietjen WJ, Rovner JS. 1980. Trail-following behavior in two species of wolf spiders: sensory and etho-ecological concomitants. *Anim Behav.* 28:735-741.

Tietjen WJ, Rovner JS. 1982. Chemical communication in lycosids and other spiders. In: Witt PN, Rovner JS, editors. *Spider communication: mechanisms and ecological significance*. Princeton: Princeton Univ Press. pp 249-280.

Tobias M, Murphey RK. 1979. The response of cercal receptors and identified interneurons in the cricket (*Acheta domesticus*) to air streams. *J Comp Physiol* 129:51-59.

Trabalon M, Bagneres AG, Roland C. 1997. Contact sex signals in two sympatric spider species, *Tegenaria domestica* and *Tegenaria pagana. J Chem Ecol* 23:747-758.

Traniello JFA. 1983. Social organization and foraging success in *Lassius neoniger* (Hymenoptera: Formicidae): behavioral and ecological aspects of recruitment communication. *Oecologia* 59:94-100.

Traniello JFA, Robson SK. 1995. Trail and territorial communication in social insects. In: Carde RT, Bell WJ, editors. *Chemical ecology of insects II.* Chapman and Hall, New York. pp 241-286.

Troyer TW, Levin JE, Jacobs GA. 1994. Construction and analysis of a data base representing a neural map. *Microsc Res Tech* 29:329-343.

Trujillo-Cenoz. O. 1965. Some aspects of the structural organization of the arthropod eye. *Cold Spr Harb Symp Quant Biol.* 30:371-382.

Trujillo-Cenoz O. 1985. The eye: development, structure and neural connections. In: Kerkut GA, Gilbert LI, editors. *Comprehensive insect physiology, biochemistry and pharmacology. V6 Nervous system: sensory.* New York: Pergamon press. pp 170-223.

Vander Meer RK, Lofgren CS, Alvarez FM. 1990. The orientation inducer pheromone of the fire ant *Solenopsis invicta. Physiol Entomol* 15:483-488.

Vassar R, Chou SK, Sitcheran R, Nunez JM, Vosshal LB, Axel R. 1994. Topographic organization of sensory projections to the olfactory bulb. *Cell* 79:981-991.

Venkateswara RP. 1963. Studies on the peripheral nervous system of the scorpion, *Heterometrus fulvipes*. Ph.D. Dissertation, Sri Venkateswara University, Tirupati, Andhra Pradesh, India.

Volknandt W, Naito S, Ueda T, Zimmerman H. 1987. Synapsin I is associated with cholinergic nerve terminals in the electric organs of *Torpedo*, *Electrophorus*, and *Malapterus* and copurifies with *Torpedo* synaptic vesicles. *J Neurochem* 49:342-347.

Waladde SM. 1982. Tip-recording from ixodid tick olfactory sensilla: responses to tick related odours. *J Comp Physiol (A)* 148: 411-418.

Walker TJ. 1957. Specificity in the response of female tree crickets (Orthoptera, Gryllidae, Oecanthinae) to calling songs of the males. *Ann Entomol Soc Am* 50:626-636.

Walthall WW, Murphey RK. 1986. Positional information, compartments and the cercal sensory system of crickets. *Dev Biol* 113:182-200.

Warburton C. 1909. Arachnida embolobranchiata: Scorpions, spiders, mites, etc. In: Harmer SF, Shipley AE, editors. *The Cambridge Natural History. V4 Crustacea and Arachnida*. London: Macmillan press. pp 295-473.

Wehner R. 1987. Matched filters: neural models of the external world. *J Comp Physiol (A)* 161:511-531.

White PR, Chapman RF. 1990. Tarsal chemoreception in the polyphagous grasshopper *Schistocerca americana*: behavioral assays, sensilla distributions and electrophysiology. *Physiol Entomol* 15: 105-121.

Whitear M. 1971. Cell specialization and sensory function in fish epidermis. *J Zool Lond* 163:237-264.

Wieczorek H, Koppl R. 1978. Effect of sugars on the labellar water receptor of the fly. *J Comp Physiol (A)*. 126:131–136.

Wieczorek H. 1980. Sugar reception by an insect water receptor. *J Comp Phsiol (A)* 138:167–172.

Wieczorek H, Shimada I, Hopperdietzel C. 1988. Treatment with pronase uncouples water and sugar reception in the labellar water receptor of the blowfly. *J Comp Physiol (A)*. 163:413–419.

Wieczorek H, Wolf G. 1989. The labellar sugar receptor of *Drosophila. J Comp Physiol (A)* 164:825-834.

Wilson EO. 1959. Source and possible nature of the odor trail of fire ants. *Science* 129:643-644.

Wilson EO, Bossert WH. 1963. Chemical communication among animals. *Recent Progress in Hormone Research.* 19:673-716.

Wolbarscht ML, Dethier VG. 1958. Electrical activity in the chemoreceptors of the blowfly. I. Responses to chemical and mechanical stimulation. *J Gen Physiol* 42:393–412.

Yetman S, Pollack GS. 1986. Central projections of labellar taste hairs in the blowfly, *Phormia regina* Meigen. *Cell Tissue Res* 245: 555-561.

Young D. 1989. Nerve cells and animal behavior. Cambridge: Cambridge Univ Press.

Yunker CE, Andrew HR, Norval RAI, Keirans JE. 1990. Inter- specific attraction to male-produced pheromones of two species of *Amblyomma* ticks (Acari: Ixodidae). *J Insect Behav* 3:557-567.

Zhao H, Ivic L, Otaki JM, Hashimoto M, Mikoshiba K, Firestein S. 1998. Functional expression of a mammalian odorant receptor. *Science*. 279:237-241.